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NOTES ON THE FAUNA ABOVE MUD BOTTOMS IN DEEP WATER IN THE GULF OF MAINE[1]

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Introduction

It has long been known that the waters just above bottom, in the mud-floored depressions of the western side of the Gulf of Maine at depths of 70–90 meters, are the home of an abundant population of the large edible shrimp, *Pandalus borealis*, accompanied by the smaller species, *P. montagui*, *P. propinquus* and *Dichelopandalus leptoceros* (Rathbun, 1883, p. 142; 1884, p. 819). After the introduction of the otter trawl to the Gulf of Maine waters, small amounts of these shrimps were landed from time to time in New England ports, and experimental trawling by the General Sea Foods Corporation in 1927–1928 again revealed the presence of *P. borealis* and its companion species in commercial quantities in the deep bowl just north of Cape Ann (Birdseye, 1928). But exploitation failed to follow, although Hjort's explorations of 1897 and 1898 (Hjort and Ruud, 1938) had long before led to the development of an extensive fishery in the other side of the Atlantic, which has gradually expanded to all suitable localities around the coasts of Norway.

Investigations by Hjort and Ruud (1938) and by Broch (1935) had also shown that *P. borealis* is but one member of a distinctive faunal community, some of the members of which live on bottom, others in the waters close above. Information from various sources had similarly shown that the *Pandalus* of the Gulf of Maine have as companions the same species of flat fishes, of rose fish (*Sebastes*), and of rockling (*Enchelyopus*), as in Norway, as well as several gadoids (*Merluccius*, *Urophycis*), which while peculiar to American waters, are at least similar to gadoid members of the Norwegian shrimp community. Hjort and Ruud's successive explorations had further demonstrated that *Pandalus* occurs in greatest abundance in situations where the bottom waters are sufficiently quiescent for organic debris to settle to the bottom.

[1] Contribution No. 210 from the Woods Hole Oceanographic Institution.

The presence of similar faunal assemblages living under conditions presumably the same in the two sides of the Atlantic opens interesting problems of environmental control. The Woods Hole Oceanographic Institution, therefore, welcomed the opportunity, in August, 1936, to make a further survey of the Gulf of Maine shrimp grounds with "Atlantis," under Professor Hjort's personal supervision, and with the assistance of Captain Hagbart Høium, an experienced Norwegian shrimp trawler.

The purpose of the investigation being primarily to explore the abundance of *Pandalus* and of associated fishes in a favorable environment, most of the hauls were made in the bowl north of Cape Ann and west of Jeffrey's Ledge (Area *A*, Fig. 1), where shrimps were already known to be plentiful, and which resembles the Norwegian shrimp grounds in its fjord-like topography and considerable depth. For comparative purposes, hauls were also made in the northeastern and southwestern branches of the open basin of the Gulf (Areas *B, C*, Fig. 1). Corings of the bottom for chemical analyses were obtained at thirteen of the stations.

The catches were so encouraging from the fisheries standpoint, that the exploration of the local shrimp stock was continued during the following two months by local fisheries interests—again under the supervision of Professor Hjort and of Captain Høium.

The present report on the regional distribution of the more prominent members of the *Pandalus* community is a sequel to Hjort and Ruud's (1938) preliminary report, and to Walford's (1936) discussion of the prospects of the shrimp fishery that appears now to be in the process of development.

Methods

The hauls (with two exceptions) were made with otter trawls of the sort used in the Norwegian shrimp fishery, brought by Professor Hjort from Norway for the purpose, the distinctive feature of which is that the footrope works about six inches above the bottom to avoid catching mud, with a sweep rope suspended below (for detailed description, see Walford, 1936). The success of the cruise was largely due to Captain Høium's skill in their use. One trial was also made with the footrope scraping the bottom. The mesh-size of the Norwegian nets was 1¾ inches (stretched) throughout. But four sizes of trawl were employed, their footropes respectively 35, 76, 82, and 117 feet in length, and the hauls varied in duration from 30 to 90 minutes, at an average speed of 1.5 knots. It has therefore been necessary to adjust the catches to a common standard, for comparative purposes,

a 60-minute haul with 82-foot trawl being chosen as the procedure most often followed during the cruise. Unfortunately, this adjustment is only a rough approximation, because the precise outline assumed by an otter trawl depends on a variety of factors such as speed, angle of the

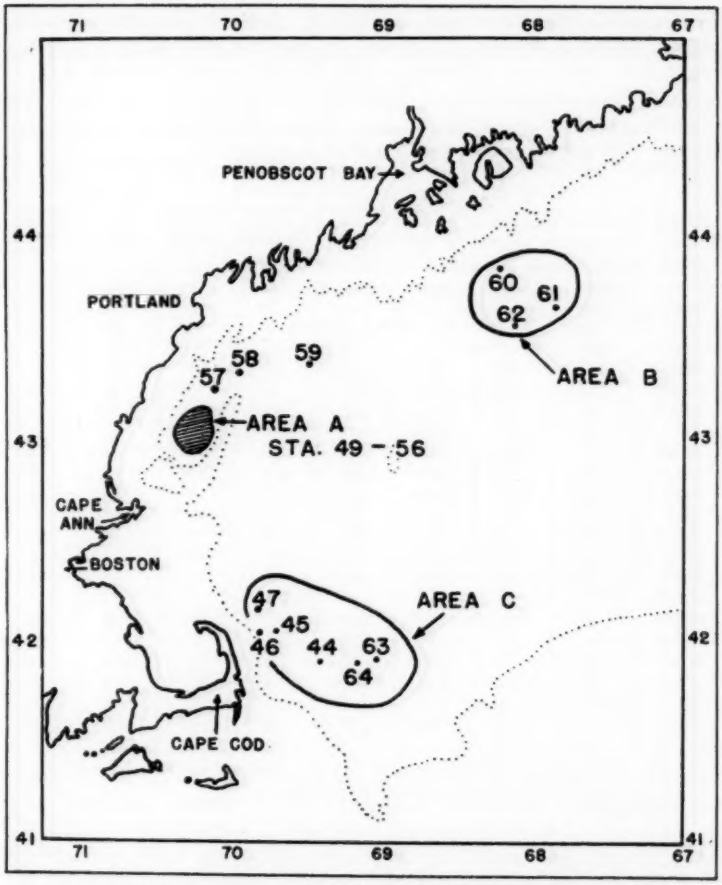

FIG. 1. Chart of western part of the Gulf of Maine, showing locations of "Atlantis" stations 2644–2664, August 18–23, 1936.

boards, and the type of bottom over which it is being drawn. The present calculation is based on the assumption that the breadths of sweep of trawls with footropes of different lengths are in the same ratios as semicircles of corresponding perimeters.

The chemical procedure, for which our thanks are due to Dr. S. A. Waksman, consisted of analyses of the upper half-inch of each core, for carbon, by the chromic acid oxidation method, and for total nitrogen by a semi-micro-Kjeldahl method. These were carried out at the New Jersey Agricultural Experiment Station at New Brunswick, New Jersey. Measurements were also made at Woods Hole of percentage loss on ignition, but showed considerable irregularity. Since this loss does not

TABLE I

Catches of the more prominent species at each station, adjusted as above (p. 306). Numbers italicized are derived from approximate counts.

Station	Depth	Amounts of Pandalus in liters	Numbers of Sebastes	Numbers of Merluccius	Numbers of Hippoglossoides	Numbers of Glyptocephalus	Numbers of Urophycis tenuis	Numbers of other fish‡
2644	200	5	*108*	15	0	0	11	6
2645	183	2	16	8	0	2	3	4
2646	144	2	*432*	*240*	2	0	65	4
2647	228	1	3	1	0	0	7	11
2649	164	32	150	*700*	12	10	32	2
2650*	175	24	*224*	338	4	1	36	0
2651*	168	11	*131*	*262*	3	2	6	1
2652	160	71	*168*	*196*	13	14	39	1
2653†	155	168	107	20	47	24	28	34
2654	158	126	183	*840*	13	35	11	2
2655	168	100	*150*	*225*	13	3	40	2
2656	177	56	*283*	*351*	63	12	23	7
2657*a*	120	2	*100*	*160*	5	0	*20*	1
2657*b*	120	3	*103*	*67*	17	8	*40*	2
2658*a*	162	16	*400*	*467*	9	1	*67*	4
2658*b*	163	50	*450*	*600*	10	5	0	3
2659*a*	164	48	*150*	*600*	24	12	50	6
2659*b*	164	27	*188*	*700*	13	9	70	3
2660	192	1	250	300	26	18	100	14
2662	198	1	270	100	0	2	0	5
2663	183	4	833	*200*	5	0	47	39
2664	215	1	*67*	47	0	0	12	7

* Net damaged, calculations assume that only half the catch was saved.
† In this haul, the footrope scraped the bottom.
‡ See Table II.

represent the organic fraction alone, but also such water as is driven off from the clay fraction at high temperatures, these values are not included in the present discussion.

Qualitative Composition of the Community

The most interesting aspects of the catches from the qualitative standpoint are that the number of species taken in abundance at any given station was so small (Table I), and that the same few species

dominated, in all three areas visited, as well as at intervening locations. Among the catches of shrimps, for example, only occasional specimens of other related species, or of Pasiphaea, were noted among the more plentiful *P. borealis*. In fact, the latter so predominates among the shrimp population in deep water in the Gulf that a sample taken at random from catches made at depths greater than 160 meters in the Jeffrey's Bowl, in the summer of 1938, consisted of 738 specimens of *P. borealis*, but only 9 of all other pandalids combined. In shoaler water, however, in that general vicinity, the relationship is the reverse, as off Casco Bay in 30–40 meters, and at a neighboring station in about 90 meters, where *P. propinquus* greatly predominated over *P. borealis*

TABLE II

List of "various" fishes other than those recorded in Table I, trawled on bottom in Jeffrey's Bowl and in the open basin of the Gulf of Maine, during August, 1936.

Species	Jeffrey's Bowl	Open basin
Myxine glutinosa	x	x
Squalus acanthias	x	x
Raia senta	x	
Raia stabuliforis	x	x
Raia scabrata	x	x
Clupea harengus	x	x
Artediellus uncinatus	x	
Cyclopterus lumpus	x	
Lumpenus lampetraeformis	x	
Cryptacanthodes maculatus	x	x
Pollachius virens		x
Gadus callarias	x	x
Melanogrammus aeglefinus		x
Urophycis chuss	x	x
Urophycis chesteri		x
Enchelyopus cimbrius	x	x
Brosme brosme		x
Macrourus bairdii		x
Lophius americanus		x

(39 to 0 and 39 to 1 respectively) at that same time. This is in line with Rathbun's (1883, p. 143) statement that while *P. montagui* and *Dichelopandalus leptoceros* are often associated with *P. borealis* over soft bottoms, they are not only the most plentiful in shoaler water, but occur on gravelly and sandy bottoms as well as on mud.

Our catches of fishes, in August, 1936, were similarly dominated by a group of 5 species, in combination, namely: by rose fish (*Sebastes marinus*), silver hake (*Merluccius bilinearis*), hake (*Urophycis tenuis*) and flat fishes (*Glyptocephalus cynoglossus* and *Hippoglossoides platessoides*), and to such an extent that the total catch of about 11,900 fish

included only 183 specimens of all the other species combined, that are included in Table II. The total list of 24 species together with a few others that have been picked up in deep trawlings previously (*Argentina, Triglops, Cottunculus, Aspidophoroides, Liparis, Pholis, Leptoclinus, Zoarces, Lycenchelys*) includes the great majority of the members of the Gulf of Maine fish fauna that are to be expected on the floor of the deep troughs, whether as regular inhabitants or stragglers. This is a very much less varied assemblage than inhabits the corresponding depth zone along the offshore slope of the Gulf of Maine and of southern New England (for complete list of records there, see Goode and Bean, 1896).

Regional Distribution

Regional segregation of the catches (Table III) shows that shrimps were taken in much greater average abundance in the deepest part of the Jeffrey's Bowl (Area *A*) and on the northern slope of its northern rim (Station 2658, 163 meters) than in either branch of the main basin of the Gulf (Areas *B, C*).

TABLE III

Regional distribution of average catches.

Area	Hauls	Shrimp	Sebastes	Merluccius	Flat fish	Urophycis	Various fishes	Total fishes
	Number	Liters	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers
A	7	60	187	416	28	26	2	649
B	2	1	260	200	23	50	10	543
C	6	2	243	92	2	34	12	383
St. 2658	2	33	425	534	13	0	5	977

From the environmental standpoint, the outstanding feature of this Bowl is the fjord-like character given to its deepest parts (170–192 meters), by the shallow ridge of Jeffrey's Ledge to the south and offshore, and on the north, by a sill with a maximum depth of about 134–137 meters (Fig. 2). This enclosure appears to be more effective, so far as concerns the activity of circulation of the bottom waters, than the topography of the sill might suggest, for temperature averages some 1–2° colder in midsummer in the bottom of the trough than at corresponding depths in the open basin to the east of the barrier, only a few miles distant.

Hjort and Ruud (1938, p. 103) have already pointed out that the mud was richest in carbon and in nitrogen where large catches of shrimps were made. This is corroborated by the following comparisons of the catch of shrimps with the percentages of organic carbon and of nitrogen

in the corings (Table IV), the average being 4.02 per cent of organic matter for the group of stations where the catch of shrimps was more than 30 liters, but only 3.05 per cent for stations yielding 5 liters of shrimps, or less.

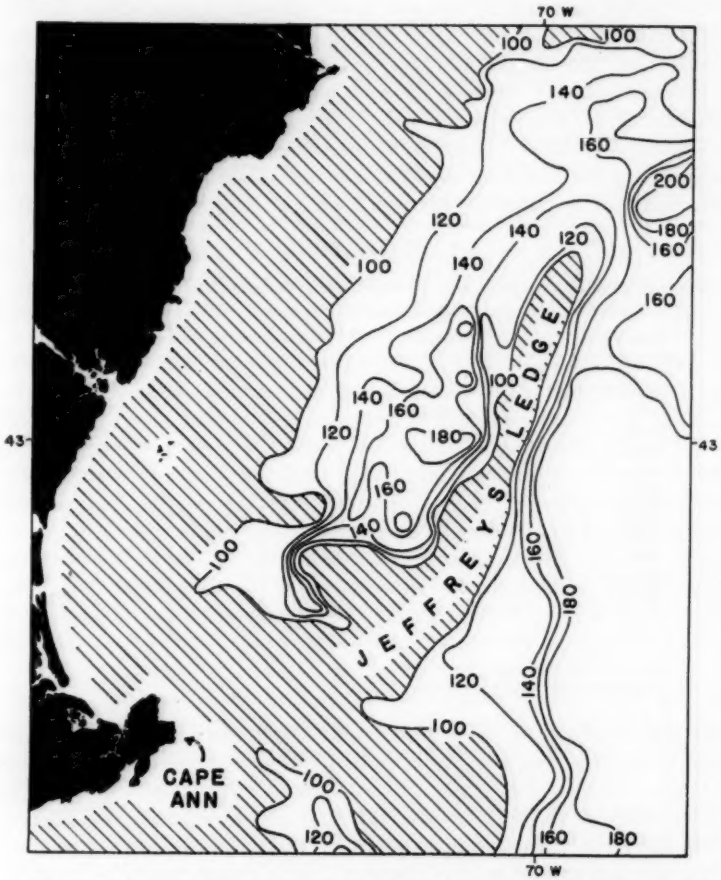

FIG. 2. Submarine topography of the region between Jeffrey's Ledge and the western coast-line of the Gulf of Maine at depths greater than 100 meters.

At one station (2662), it is true, where the percentage of organic matter was high, the catch of shrimps was very small; regional irregularities are, in fact, to be expected, in the nature of the case. But in no instance was the mud poor in organic content where shrimps were

abundant. While the correlation is not so close between the catches of shrimps and the percentage of nitrogen, the latter averaged 0.338 per cent for the rich and 0.234 per cent for the poor shrimp stations. In other words, the average percentage of organic carbon was about one-third higher, and that of nitrogen about one-half higher where catches of shrimp were relatively large than where they were small. Unfortunately, the data do not afford so satisfactory a comparison from the regional standpoint, because the mud was sampled at only two of the stations in the deeper part of the Jeffrey's Bowl. At each of these (Stations 2649, 2654), however, the percentage of organic carbon closely approached the maximum found at any station, while the percentages of nitrogen (0.295 and 0.309 per cent) were at least somewhat above the

TABLE IV

Percentage of organic matter and of nitrogen in dry mud and catch of *Pandalus*.

Station	Shrimps	Organic matter*	Nitrogen
	liters	*per cent*	*per cent*
2654	126	4.1	0.309
2659	37 av.	3.7	0.195
2658	33 av.	4.2	0.553
2649	32	4.1	0.295
2644	5	2.6	0.311
2663	4	2.6	0.115
2657	2.5 av.	2.4	0.201
2645	2	3.7	0.217
2647	1	3.1	0.205
2660	1	3.3	0.301
2662	1	4.0	0.310†
2664	1	2.8	0.217

* Carbon X 1.724.
† Determination made at Woods Hole.

average for the series as a whole (0.262 per cent). It is interesting that the station that was the richest of all, both in organic carbon and nitrogen (Station 2658) was at least moderately productive of shrimps, though located north of the sill. Thus, it seems sufficiently established, both for Norwegian waters and for the Gulf of Maine, that in summer *Pandalus* is abundant only in situations where organic matter is plentiful on the sea floor below; and by available evidence this applies to the males throughout the year. But the fact that considerable catches of ovigerous individuals were landed by fishermen from depths of 70–90 meters only, in the northwestern side of the Gulf, during the winter of 1938 suggests that the large females tend to work inshore into shoaler and more turbulent situations during the egg-bearing period.

It seems that we are dealing here with a food relationship, for while no direct observations are available as to the diet of *Pandalus* in the Gulf of Maine, Wollebaeck (1903, see also Hjort and Ruud, 1938, p. 60) found the stomachs of this shrimp in Norwegian waters to contain animal remains of various origins, among a mass of indeterminate mud, evidence that while it feeds on bottom, its diet consists of the remnants of dead organisms or perhaps of bacteria and protozoa, rather than of the mud itself. This is in line with Waksman's (1933) demonstration that the organic matter in the mud is largely in the form of humus, and hence not likely to be readily digestible by the higher animals.

The catches of *Merluccius* also averaged considerably larger in Area *A* and at the neighboring station (2658) than in either of the other areas, though there is no reason to suppose that the type of bottom is of direct influence in this case, but rather that the significant parallelism is between the number of fish and of shrimps, as discussed below. *Sebastes,* on the other hand, averaged somewhat less plentiful in the Jeffrey's Bowl (Area *A*) than over the rim of the latter (Station 2658) or in the open basin (Areas *B* and *C*). But the difference in average catch from area to area was so small for pleuronectids and for *Urophycis* that it may not have been regionally significant.

Among the fishes of minor numerical importance, *Enchelyopus cimbrius* and *Urophycis chuss* were encountered at so many stations (respectively 13 and 12 hauls out of 22), that they appear practically universal over the deep bottoms both of the Jeffrey's Bowl and of the western branch of the open basin of the Gulf, though the maximum catch was only 9 specimens in the one case, 10 in the other. *Enchelyopus* was also taken in the eastern basin, and failure to take *U. chuss* there in our few hauls is no evidence that it does not actually occur there.

Odd specimens of all other species included in Table II, and listed on page 309, have also been taken in the bottom of the Jeffrey's Bowl at depths greater than 160 meters on one occasion or another, with the exceptions of *Triglops, Argentina, Aspidophoroides, Pholis, Leptoclinus, Brosme, Urophycis chesteri, Macrourus, Lophius,* haddock and American pollock. These are also to be expected there, either because plentiful in somewhat shoaler water in the near vicinity, or because already known to occur in deep water elsewhere in the Gulf. Arctic species in particular, such as *Cottunculus,* would be as likely to maintain a small population in the low temperatures of the Bowl as anywhere else to the west of Cape Sable.

In short, there is nothing in the available data to suggest that the sill responsible for the fjord-like character of this Bowl gives any distinctive character to the fish fauna of its bottom waters, as contrasted with

that of the open basin of the Gulf to the eastward. At most, certain species that have their center of abundance over the slope outside the Gulf (*Macrourus, Urophycis chesteri, Argentina*) may be progressively less numerous westward, until they reach the trough only as stragglers, if at all; whereas certain others may be most plentiful there, whether because of cold water affinity, or to utilize the particular food supply provided by the local abundance of *Pandalus*, as appears to be the case with *Merluccius*.

Urophycis tenuis has long been known to feed chiefly on shrimp (see Bigelow and Welsh, 1925, p. 450, for summary of its feeding habits); in fact, all but one of the specimens of this species that were opened during our cruise contained more or less remnants of large *Pandalus*. But the following tabulation (Table V) fails to suggest any

TABLE V

Numerical relationship of fish to shrimps.

Shrimps, liters	126	100	71	56	54	48	32	27	24	16	11	5
U. tenuis, numbers.....	11	30	40	26	0	50	34	75	36	67	6	23
Sebastes, numbers.....	183	150	168	283	450	150	150	188	224	400	131	108
Flat fish, 2 sp., numbers..	48	16	25	75	15	36	22	22	6	10	5	0
Merluccius, numbers.....	840	225	196	351	600	600	700	700	338	467	262	15

Shrimps, liters	4	3	2	2	2	1	1	1	1
U. tenuis, numbers.....	60	40	4	86	20	14	100	0	19
Sebastes, numbers.....	833	103	16	432	100	3	250	270	67
Flat fish, 2 sp., numbers..	5	25	2	2	5	44	2	2	0
Merluccius, numbers.....	200	67	8	240	160	1	300	100	47

correlation between the numbers of *Urophycis* and the amounts of shrimp taken, for the average catch of the former averaged about the same for the nine stations which yielded 24 liters or more of shrimps, as for the ten stations that yielded 5 liters or less (34 and 37 *Urophycis*, respectively), while the largest and the smallest catches of *Urophycis* were each made at a station where shrimps were very scarce.

The catches of *Sebastes* seem also to have been independent of those of shrimps, the average being almost exactly the same for the group of stations where shrimps were plentiful (20 liters or more), as for the

group where they were scarce (216 and 218 *Sebastes,* respectively); while the largest and smallest catches of *Sebastes* (833 and 3) were both made at stations yielding only 4 liters and only 1 liter of shrimps, respectively. Neither do the present data yield any information as to the extent to which *Pandalus,* when abundant, serves as prey for *Sebastes,* the great majority of the latter having regurgitated the stomach contents before they were brought on board.

A rough parallelism does, however, appear, between the abundance of *Merluccius* and of shrimps, illustrated by the facts that the catch of the former averaged four times as great (494) for the group of stations

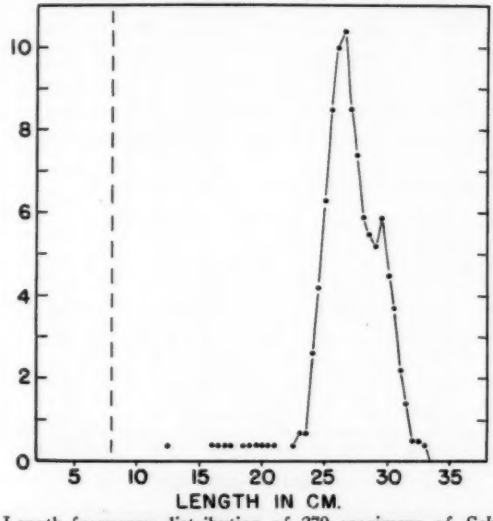

FIG. 3. Length-frequency distribution of 270 specimens of *Sebastes,* Station 2662, smoothed once by running average of three. The broken curve marks the lower limit for adequate sampling by the nets employed.

where shrimps were abundant as where they were scarce (114); that it was only where the catch of shrimps was less than 6 liters that the catch of *Merluccius* was as small as 100; and that no hauls where shrimps were so scarce yielded as many *Merluccius* as the average for the series as a whole (306). To judge from the predaceous nature of this fish and from its independence of the bottom, we are dealing here with a predator-prey relationship, though our present data fail to yield direct information in this regard for *Merluccius* any more than for *Sebastes,* and for the same reason.

Flat fishes of the two species, combined, also averaged considerably

more plentiful (av. 30 flat fish) where shrimps were in more than moderate abundance than where the catches of shrimps were 5 liters or less (av. 9 flat fish). But there being no reason to suppose that either of these pleuronectids preys to any extent on shrimps, the parallelism in this case suggests that the same muddy bottoms which provide the richest feeding for *Pandalus* are also rich in the small invertebrates on which *Hippoglossoides* and *Glyptocephalus* chiefly subsist. While we have no precise data, in this respect, for the Gulf of Maine, such is certainly true in Norwegian waters, where soft bottoms in similar situations

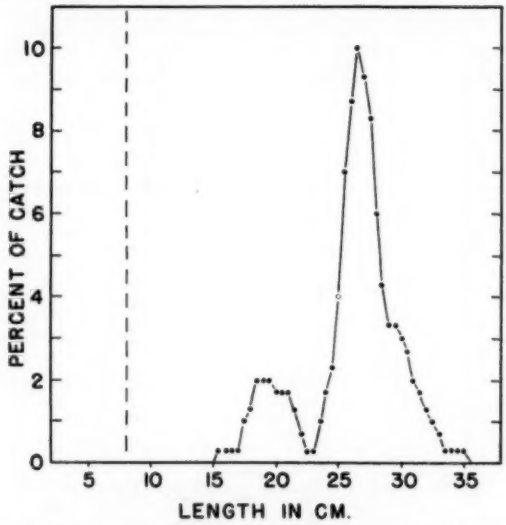

FIG. 4. Length-frequency distribution of 300 specimens of *Sebastes*, Station 2663, smoothed once by running average of three. The broken curve marks the lower limit for adequate sampling by the nets employed.

support a wealth of invertebrate life (Broch, 1935; see also Hjort and Ruud, 1938, p. 106).

Size Distribution of Different Species of Fish

It was likely due to the mesh-size of our trawls that the catches of *Pandalus borealis* consisted almost wholly of medium and large-sized individuals, the commonest lengths being from 12 to 18 cm. (rostrum-telson), which covers the upper size range for that species. But this factor cannot be invoked to explain a great scarcity, in the catches of *Sebastes*, of *Urophycis*, and of the two common flat fishes, of the smaller sizes that the mesh might be expected to sample.

In the case of *Sebastes*, the great majority were upwards of 22–23 cm. long, averaging something like 25–30 cm., a size that would be classified as "medium to large" in statistics of the commercial fishery (Figs. 3, 4). In fact, out of a total of 4,334 specimens taken, only 115 were less than 20 cm. long, and only 56 smaller than 15 cm., although the net should certainly have sampled this spiny species adequately down to 7–9 cm., and have taken at least a scattering of still smaller sizes had they been present in numbers at all approaching those of the larger fish. The rate of growth of *Sebastes* in the Gulf of Maine has not been studied, but its pelagic larvae have been taken there up to a length of 2.1 cm. (to a considerably larger size in European waters). The facts that fry of 3.7 to 7.5 cm. are abundant on bottom in spring and summer (Bigelow and Welsh, 1925; Bigelow and Schroeder, 1936) and that in a collection made in summer at Eastport, Maine, many years ago, there is a distinct size group of 5.1–6.3 cm., make it likely that the peak at 7–9 cm. shown on Fig. 5 represents fish one year old, and the

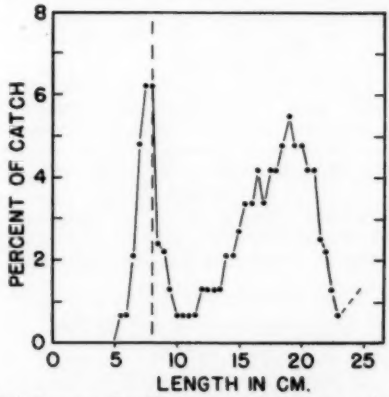

FIG. 5. Length-frequency distribution of all *Sebastes* taken less than 24 cm. long (146 specimens), smoothed once by running average of three. The broken curve marks the lower limit for adequate sampling by the nets employed.

next larger group centering at 15–21 cm., those two years old. On this basis, the fish of 24 cm. and larger, that formed the bulk of the catch in all three areas, were at least three years old and upwards. Unfortunately, our data afford no information as to how many year classes were actually included, for these older fish appear as a single size-group on the graphs for the two stations where unselected samples were measured (Figs. 3, 4, Areas *B, C*), while inspection of the fish larger than 20 cm. in Area *A*, as brought on deck, showed that there was no obvious succession of recognizable size-groups among them, either. In other

words, it is likely that had the entire catch of more than 4,000 specimens been measured, the resultant graph would have closely resembled Figs. 3, 4. However, since the dominant group includes the sizes that yield commercial catches, we may assume that the greater part of the usual life of the species is included from three years onward—however long that may endure. In fact, occasional specimens of 43–45 cm. are as close an approach to the maximum yet recorded for the Gulf (about 60 cm., Bigelow and Welsh, 1925, p. 305) as was to be expected from so few hauls.

The absence of small sizes is still more striking for *Urophycis tenuis,* for not a single specimen shorter than 21 cm. was taken among a total of 664, ranging up to about 70 cm. in length and to 8 lb. in weight, though the net could be depended upon to take them down to 16–17 cm. Again, in the cases of *Hippoglossoides* and *Glyptocephalus,* the smallest sizes (within the sampling range of the nets, or down to 10–15 cm.)

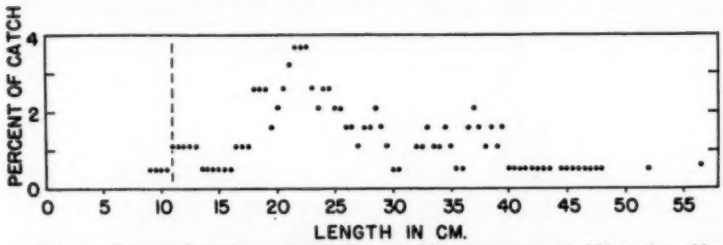

FIG. 6. Length-frequency distribution of 187 specimens of *Hippoglossoides platessoides,* smoothed once by running average of three. The broken curve marks the lower limit for adequate sampling by the nets employed.

were much less strongly represented than the successively larger sizes (Figs. 6, 7). In fact, *Hippoglossoides* yielded only 21 specimens shorter than 20 cm., and *Glyptocephalus* 17, out of totals of 183 and 135 respectively. If previous estimates of the rates of growth of these two pleuronectids in the Gulf of Maine be correct (Bigelow and Welsh, 1925, pp. 486 and 515; Bigelow and Schroeder, 1936, pp. 340–341), the first size-group of each that was well represented in the catches, centering at about 17–20 cm. for *Glyptocephalus* and at 20–23 cm. for *Hippoglossoides,* was at least two years old—with the yearlings only about one-fourth to one-third as numerous. Or, if Molander's (1925) estimate, based, however, on conditions in the Baltic, applies, the first prominent group of *Glyptocephalus* would be the four-year-olds. The evidence afforded by these two species is especially instructive, because the generality of distribution over the mid-range of included sizes shows that the local stocks had not recently been subject to any outstanding variations in the success of individual year classes.

In short, our catches contain a very much weaker representation of the first two or more year classes that the nets might be expected adequately to sample of *Sebastes,* of *Urophycis,* and of the two pleuronectids than of the older fish, whereas the reverse would be the expectation in a normal population distribution. Since this applies to four species of widely separated taxonomic groups, differing widely, too, in their life histories, it is safe to assume that we have to do with a general depth or regional relationship, not with a chance irregularity in the relative strengths of individual year classes, such as might have been argued had only one species been at issue. If, then, the year 1936 be acceptable as representative, it appears that this group of fishes does not disperse into the deep troughs of the Gulf until they are at least two or more

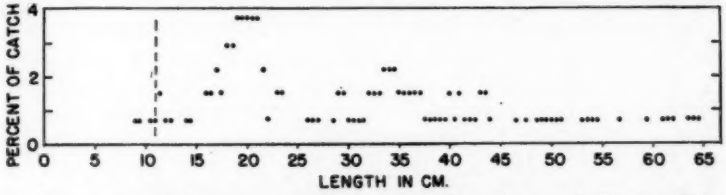

FIG. 7. Length-frequency distribution of 135 specimens of *Glyptocephalus cynoglossus,* smoothed once by running average of three. The broken curve marks the lower limit, for adequate sampling by the nets employed.

years old, a conclusion at least compatible with their generally non-migratory nature. The graphs further suggest that such individuals of these species as do stray out into these situations, or descend thither from the upper strata, tend, on the whole, to remain there year after year—or at least that none of these species shows any general tendency to depart thence, with increasing age. This appears also to apply to the few cod that wander out onto these deep soft bottoms, where very large specimens are sometimes caught, but very few small ones.

In the case of *Merluccius,* however, the catches were strongly dominated by a size group, with a modal length of about 17 cm., with the next longer (and very much less abundant) group centering at about 26 cm. (Fig. 8). Earlier studies (Bigelow and Schroeder, 1936, p. 338) would suggest that the first of these groups represents the fish hatched in the preceding summer, i.e., the yearlings, the second size group being the two-year-olds. Confirmation of this growth schedule for these comparatively cold waters is afforded by the fact that a small lot (28 specimens) taken near Mt. Desert in July, 1930, fell into the same approximate size divisions (15 fish, average, 15 cm.; 13 fish, average, 26 cm.) with no fish between 20 and 23 cm. in length. *Merluccius* of 18–21 cm. taken near Chatham in the same season may best be interpreted as

one-year-olds that had grown faster at somewhat higher temperatures. This leaves only the post larvae of the year unaccounted for, among the younger fish. These would be so small (perhaps 2.5–3.5 cm.) in the month in question—if indeed they descend into the bottom waters by that date—that our nets would not have taken them; nor would have until after several months more growth on their part, to judge from the fact that fry trawled by "Albatross II," off Long Beach, New York, in 1928 were only 5.7–11.8 cm. long in November. Thus, *Merluccius* (unlike its companion species) passes its first summer of rapid growth (second summer of life) in great abundance in deep water. The dis-

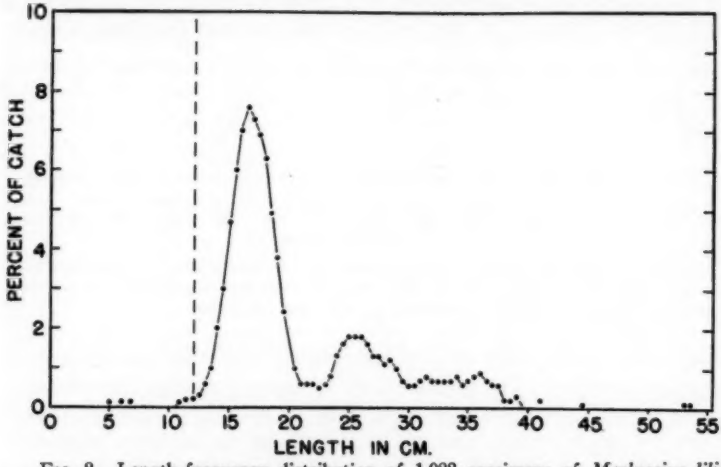

FIG. 8. Length-frequency distribution of 1,088 specimens of *Merluccius lilinearis*, smoothed once by running average of three. The broken curve marks the lower limit, for adequate sampling by the nets employed.

covery of this habitat for it, combined with captures of this same size group in 18–22 meters near Mt. Desert, and of fish slightly larger in 45–50 meters off Cape Cod (mentioned above), is especially interesting, because previously this age group was close to unknown in the Gulf of Maine, although larvae are produced there in profusion, and larger fish of 20 cm. and upwards occur there in great abundance, both inshore and on the Banks (Bigelow and Welsh, 1925, p. 389). Present findings thus corroborate the earlier suggestion that young *Merluccius* are, in fact, as plentiful in the Gulf as the local abundance of adults would suggest, but that they keep to somewhat deeper water for the most part.

It appears, however, that most of the juvenile *Merluccius* that find their way out, or down, into the troughs, remain there through one summer of growth only, else our hauls might have been expected to

have yielded a much larger proportion of the next larger, and of subsequent size groups than was actually the case. Whether their subsequent departure takes place in the following autumn as part of the migration out of the Gulf of Maine, with which the silver hake is generally credited (Bigelow and Welsh, 1925, p. 388), or whether the troughs actually serve as wintering grounds for at least a part of the Gulf of Maine stock, must remain an open question, until these localities are explored during the cold months. We may, however, point out that the latter alternative would at least be compatible with the temperature cycle. The water at 150–170 meters is coldest in spring and summer, both in the Jeffrey's Bowl (2.5–4.5°) and in the western branch of the western basin (4.5–6°), warmest in late autumn or early winter (5–7° and 6–8°, respectively), while deeper still, in the basin the seasonal range is normally less than 1.0° (see Bigelow, 1927, Figs. 5, 6, 70, 72).

In any case, it now seems established that in subsequent feeding seasons most of the *Merluccius* that inhabit or visit the Gulf, repair to shoaler waters either on the Banks or around the coastal slope, to prey on the young fry of various other fishes.

Amounts of Pandalus and Fish Combined

Finally, speculation is perhaps permissible as to the total mass of animals, of a size our nets would sample, that exists in summer in the water next to bottom, in the rich shrimp area in the Jeffrey's Bowl. In this connection we have first to consider how much (if at all) the catches were impoverished by the failure of the nets to strain the water immediately in contact with the bottom. Our only direct evidence in this respect is the difference between catches in the standard hauls made with raised footrope, and in the one haul in which the footrope swept bottom (Station 2653), which yielded at the rate (adjusted) of 107 *Sebastes*, 20 *Merluccius*, 71 flat fish, 33 *Urophycis*, 34 " various " fishes, and 168 liters of *Pandalus*. This contrasts with an average of 377 *Sebastes*, 713 *Merluccius*, 32 flat fish, 49 *Urophycis*, 7 " various " fishes, and 99 liters of shrimps, for the three most productive catches of each of these categories with raised footrope, and with a maximum of 450 *Sebastes*, 846 *Merluccius*, 75 flat fish, 86 *Urophycis*, 11 " various " fishes, and 126 liters of shrimps for any one haul of the latter type in the same general area (Area *A*, Fig. 1). On this basis, it appears that inclusion of the six inches of water next the bottom might well have raised the average catch of shrimps in this region (the richest shrimp ground) by anywhere from one-third to two-thirds. Some increase in the catch of flat fish might also have resulted; and this last is made the more probable by the fact that Birdseye's (1928) twelve experimental hauls in the same region made with trawls of the usual pattern (footrope on bottom)

yielded a somewhat greater weight of large flat fish of the two species in question (average, 275 lb.) than of shrimps (average, 217 lb.), whereas the reverse was true [2] for our hauls with raised footrope. But there is nothing to suggest that the catches of the more active and more plentiful fishes were significantly reduced by our failure to sample the few inches of water that is in immediate contact with the sea floor. If anything the reverse is suggested, perhaps because the stirring of the mud by the trawl gives the fish warning of its approach. And these three species—*Merluccius, Sebastes,* and *Urophycis*—made up the major weight of fishes caught.

Assuming that, by actually sweeping the bottom, the average catch would have been increased by 50 per cent for shrimp, and by 30 per cent for flat fishes of the two species combined, but that the catches of the other major species of fish would not have been materially affected, the average for the Jeffrey's Bowl, deeper than 150 meters would be 37 flat fish, 187 *Sebastes,* 416 *Merluccius,* 26 *Urophycis,* 2 odd fishes, and 90 liters of *Pandalus.* Crediting the latter with an average weight of 0.97 lb. per liter as determined by measurements made on board, and using conversion factors of 1 lb. per fish for *Sebastes,* 0.25 lb. for *Merluccius,* 1 lb. for each of the pleuronectids, and 6 lb. for *Urophycis tenuis,* as derived from examination of the catches, and perhaps 1 lb. for the odd fishes of other species, we arrive at a general average weight of some 576 lb. per standard haul, for a water layer perhaps two meters in thickness. This corresponds to 55 lb. of fish and shrimps, above each acre, on the assumption that the breadth of the strip fished by the 82-foot trawl was about 52 feet, and that the distance covered averaged 1.5 sea miles per haul.

We should emphasize that if the foregoing assumptions even approximate the truth, the resultant weight is minimal, because it is based on the further assumption that our nets, in their passage, would capture every individual shrimp, and every fish of appropriate size that lay in their paths. We may well leave this discussion at this point, because no definite information is available as to what proportion of the more active fishes would be likely to dodge the trawls, or to what extent this potential loss would be counterbalanced by the opposing tendency for the lead-ropes to the otter boards to turn inwards, toward the mouth of the net, such other fishes as might be to either side of the latter.[3]

[2] Crediting the *Glyptocephalus* and *Hippoglossoides* with an average weight of 1 lb. each.

[3] For discussion of the relative efficiency of trawls rigged in this way—the so-called "V.D." system—as compared with those with the boards close in to the net itself, see Hickling, 1931.

Summary

Findings from 22 trawl-hauls, at 20 stations on mud bottoms in the western and northern parts of the Gulf of Maine, in depths between 120 and 228 meters, August, 1936, are:

1. The faunal community of the water just above these deep mud bottoms is so monotonous that the catch consisted, in every case, almost wholly of five species of fish (*Sebastes marinus, Merluccius bilinearis, Urophycis tenuis, Hippoglossoides platessoides, Glyptocephalus cynoglossus*) and of the shrimp, *Pandalus borealis,* in varying combinations of relative abundance. The only exception was at one station where a larger catch was made of crabs (*Geryon*) than of shrimp.

2. The only other fishes taken were odd examples of the 19 species listed on page 309. The total number of species to be expected in such situations (except as accidental strays) is probably not more than 35.

3. As the nets were arranged to fish off bottom, benthonic invertebrates were not sampled.

4. The most significant faunal contrast, from the regional standpoint, is that *Pandalus* has a well-marked center of population, at least in summer, in the deep bowl west of Jeffrey's Ledge where bottom currents are weak. A positive correlation was also shown between the amounts of shrimps caught at different stations and the percentage of organic matter in the mud, corroborating Hjort and Ruud's (1938) earlier conclusion that *Pandalus* is abundant only where the supply of organic debris on the bottom is relatively large.

5. *Merluccius* was also most plentiful in this bowl, probably in pursuit of shrimp. But the catches do not support any prevailing contrast between this locality and the open basin of the Gulf, in the abundance of any one of the other four fishes of which large catches were made. Neither is any regional contrast suggested in the qualitative composition of the fish fauna over mud bottoms in different parts of the Gulf at the depths in question.

6. The stock of *Merluccius,* in the localities and depths studied, was strongly dominated by the yearlings, an age group the habitat of which in the Gulf of Maine was previously unknown, though the older fish are extremely plentiful there in shoaler water. But the catches of *Sebastes,* of *Hippoglossoides,* of *Glyptocephalus,* and of *Urophycis tenuis* consisted chiefly, or (in the last case) wholly of fish of two years and upwards. The few cod that have been taken in the bowl from time to time have all been very large, though smaller cod as well find their way down into the deeps of the open basin of the Gulf.

7. Length-frequency distribution of the catches added to previous information, indicates that *Sebastes,* in the Gulf of Maine, attains an average length of about 7.5 cm. at one year of age and of about 18 cm.

at two years. But subsequent growth is so slow that age-analysis of larger sizes would require a larger series of measurements than are yet available. Yearlings of *Merluccius* average about 17 cm. by late summer in the deep waters of the Gulf, the two-year-olds about 25 cm.

8. It is estimated that the combined weight of shrimp and fish of all species, in the bowl west of Jeffrey's Ledge, at depths greater than 150 meters, would have averaged at least 576 lb. per haul of 1.5 sea miles with an 82-foot trawl, or 55 lb. per acre of bottom, had the 6-inch stratum next the sea floor been included.

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TASTE THRESHOLDS IN LEPIDOPTEROUS LARVAE

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Introduction

The first attempt to measure the taste thresholds of lepidopterous larvae was made on eleven species including *Liparis dispar* L., *Danaus plexippus* L., *Papilio ajax* L., *Diacrisia virginica* Fabr., *Estigmene acrea* Dru., *Platysamia cecropia* L., and *Isia isabella* A. & S. (Dethier, 1937). The threshold concentrations were found to be approximately eight times less dilute than those of man. Eger (1937), working with several European species, obtained results almost identical for HCl but could get no definite reactions with sugar solutions.

The present paper represents an attempt to ascertain more accurately the threshold values for other substances in additional species. It also attempts to show that larvae of *Malacosoma disstria* Hbn. and *Apamea velata* Wlk. are sensitive to sugar solutions and that reasonably accurate threshold values can be determined.

Methods

Two species were tested, *Malacosoma disstria* Hbn. and *Apamea velata* Wlk. A few tests were also conducted with *M. americana* Fabr. In the case of *M. disstria* experiments were carried on in the field under natural conditions. Larvae were imprisoned upon the trunk of their food-tree between two encircling bands of Tanglefoot which prevented them from ascending or descending the trunk. In addition each animal was marked with a daub of paint. Thus the gastronomic history of each could be followed. Solutions to be tested were offered on the end of a fine glass rod. This method of experimenting with larvae in the field is most advantageous since such external factors as temperature and humidity are those of the natural environment of the animals. In addition, excessive handling is eliminated. Unfortunately such methods are not applicable to *A. velata* which lives close to the ground on low-lying herbage. In this case the experimental animal was allowed to crawl on a clean ground glass plate. A drop of the solution to be tested was then placed in the path of the larva. This general experimental procedure was also followed with a few specimens of *M. disstria* to

serve as a check against possible differences arising from the dissimilarity of the two procedures. Although it was shown that such differences did not arise, a few larvae living under laboratory conditions sickened and died while all those in the field eventually pupated.

All larvae were offered distilled water and those which accepted were eliminated from experiments with sugars. When testing with HCl only those animals were used which accepted water. Following this preliminary determination drops of the test solution were offered. Each test was immediately preceded and followed by a trial with distilled water which served as a control and as a means of cleansing the mouthparts. The order of presentation of solutions was constantly changed in order to exclude the possibility of association.

Thresholds are stated in terms of two concentrations indicating in the case of sugars that the animals refused the lower and accepted the higher; in the case of acids, that the animals accepted the lower and refused the higher. The lowest concentration detectable by the larva lies between these two values.

There were two possible sources of error, first that arising from diluting the stock solution, second that due to evaporation after the solutions had been made up. The latter source was eliminated to a large extent by making up fresh solutions directly preceding each series of experiments. The deviation from the calculated molarities in a series of sugar dilutions due to both of the above-mentioned sources combined was determined twice by measuring the specific gravity, first, directly upon the conclusion of a series of experiments. The deviation from the calculated molarities was $\pm$ 0.9 per cent. Second, a similar check was run on a series of solutions thirty days old. The determined values were now 15 per cent higher. This illustrates the need of working with fresh solutions. All the threshold values stated are the determined values. Sugar solutions were made from Merck and Mallinckrodt purest sugars with distilled water as a solvent. New unused glassware was employed and there was no evidence of bacterial contamination.

HCl

One hundred and thirty-eight tests were made with *M. disstria* and thirty with *A. velata*. Different molarities forming the following series were made up by careful dilution and offered to the larvae: .001M, .002M, .003M, .004M, .005M, .01M, .02M, .03M, .04M, .05M, .06M, .07M, .08M, .09M, .1M. The lowest threshold value obtained lay between .004M and .005M for both species. The lowest found by Eger lay between .003M and .006M. Dethier (1937) found a value between .0005M and .005M. The average threshold value for *M. disstria* was

.018M and .0085M for *A. velata.* Figure 1 illustrates clearly the difference in sensitivity to HCl between the two species. Though the lowest threshold value obtained was the same for both, it may be seen from the graphs that there was a greater percentage response to .005M HCl by *A. velata* than by *M. disstria.* A comparison of responses to .01M HCl reveals that the percentage response of *A. velata* at that concentration was just twice that of *M. disstria.*

Throughout the experiments *A. velata* larvae proved most difficult subjects. Occasionally there were feeble responses to .004M HCl but these were very doubtful. The average threshold was the same for

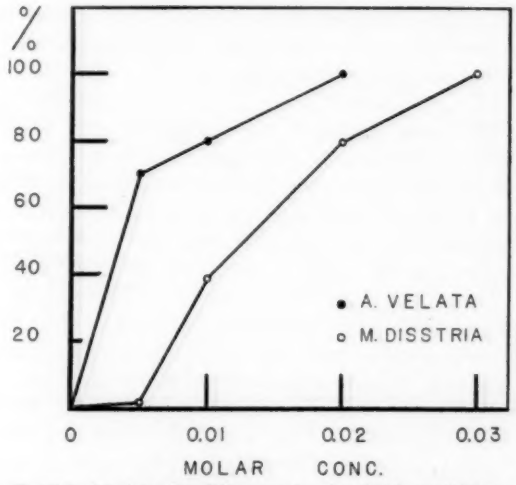

FIG. 1. Graphs showing the difference in threshold sensitivity of *A. velata* and *M. disstria* to HCl. The percentage response is plotted on the ordinate and the molar concentration of HCl on the abscissa.

first instar larvae as for last instar larvae of *M. disstria.* Indications are that a period of starvation lowers the acceptance threshold, but more work is necessary to confirm this.

Sucrose

Three hundred and nine tests were made with *M. disstria;* eighty tests, with *A. velata.* The following sucrose molarities were employed: .008M, .01M, .02M, .03M, .04M, .05M, .06M, .07M, .08M, .09M, .1M, .125M, .2M, .250M, .3M, .4M, .5M, .6M, .7M, .8M, .9M, 1M. The lowest threshold value obtained lay between .01M and .02M for *M. disstria,* between .01M and .02M for *A. velata.* The average threshold

value was .015M for *M. disstria,* .037M for *A. velata.* Again it was possible to detect no change in the average threshold value for larvae at different instars.

Fructose

Eighty-one tests were made with *M. disstria;* eighty-two, with *A. velata.* Tests were made with the following molarities: .008M, .01M, .02M, .03M, .06M, .0625M, .1M, .125M, .2M, .250M, .3M, .4M, .5M, 1M. For *M. disstria* the lowest threshold value obtained lay between .03M and .06M; for *A. velata,* between .01M and .02M. The average

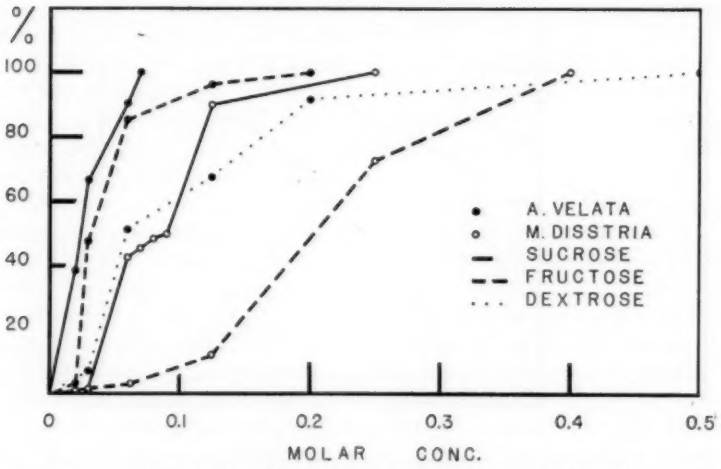

FIG. 2. Graphs showing the difference in threshold sensitivity of *A. velata* and *M. disstria* to various sugars and the order of effectiveness for these sugars. The percentage response is plotted on the ordinate and the molar concentration of the sugars on the abscissa.

threshold for the former was .273M, .058M for the latter. No age effect was demonstrable.

Dextrose

Sixty-eight tests were conducted with *A. velata.* Solutions from the following series were tested: .008M, .01M, .02M, .03M, .06M, .0625M, .1M, .125M, .2M, .250M, .5M, 1M. The lowest threshold value obtained lay between .02M and .03M. The average threshold value was .138M. *M. disstria* larvae failed to respond to dextrose even as concentrated as 1M.

Conclusions

On the whole, *A. velata* appeared more sensitive to taste substances than *M. disstria* (see Fig. 2). The order of sensitivity to the various sugars was the same for both species, namely, sucrose, fructose, dextrose, and lactose, in decreasing order of sensitivity. It was possible to demonstrate but a small response to maltose and none at all to lactose. *M. disstria* failed to respond to dextrose. *M. americana,* with three exceptions, failed to respond to any sugars. The three animals which did respond did so to .1M sucrose. Larvae which responded to sucrose concentrations as low as .02M refused lactose as concentrated as 1M; .1M sucrose was accepted and .1M lactose refused. Since lactose and sucrose of equal concentrations are supposed to have the same osmotic pressure and viscosity (Minnich, 1929), this indicates that responses were truly gustatory.

The previous dietary regime exercised a profound effect upon the threshold. Larvae which had been allowed to drink a quantity of sucrose always failed to respond to previously determined threshold concentrations of fructose or dextrose. Likewise an excess of fructose raised the acceptance threshold of dextrose. The reverse effect was not in evidence. This is taken as further evidence for the order of sensitivity to the various sugars mentioned above.

Although no age effect could be demonstrated (first instar larvae of *A. velata* had the same thresholds as last instar larvae), it was observed that twenty-four hours prior to pupation thresholds were exceedingly high. This condition seems to have been due to disinclination on the part of the larvae to eat rather than a rise in the threshold due to age.

Thus it may be seen that *M. disstria* and *A. velata* larvae react very definitely to sugar solutions. The values obtained for these two species compare favorably with those determined for other caterpillars.

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BUDS INDUCED FROM IMPLANTS OF NERVE CORD AND NEIGHBORING TISSUES IN THE POLYCHAETE, CLYMENELLA TORQUATA

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The problem of the influence of the nervous system on the formation of new tissues has attracted the attention of a number of workers, with perhaps the majority of them interested in its effect on the replacement of lost parts. Extensive studies in this field have been carried out on annelid regeneration with considerable divergence of reported results. By some investigators (for example, Morgan, 1902, and Bailey, 1930) it is believed that the nerve cord is essential to regeneration. On the other hand, Goldfarb (1909), Siegmund (1928), and Avel (1932) were able to obtain regeneration in the absence of any ventral nerve cord in the immediate vicinity of the cut surface. Baskin (1933) believes that the nervous system exerts an essential but entirely unspecific influence on head formation in the earthworm. Crowell (1937) says, "The evocation of morphogenesis can be directly attributed to the nerve cord or brain. The nervous structures are thus comparable to organizers." He believes, however, that, "The region of the nerve cord concerned in regeneration does not determine the form of the regenerate." In a recent paper P. L. Bailey, Jr. and G. H. Bailey (1938) report concerning regenerating tissues that, "Organization of these tissues into normal new structures in *Eisenia* and *Nereis* seems to be dependent upon the presence of the nerve cord at the cut surface and in its normal relation to the other tissues."

In an attempt to learn more about this problem the experiments reported in this paper were undertaken, first in the summer of 1934 at the Marine Biological Laboratory, Woods Hole. Each operation consisted in removing a piece of tissue from one *Clymenella* and implanting it in the body wall of the thirteenth segment of another individual of the same species. Each implant was inserted in a dorso-lateral region near the longitudinal vessel which runs in the vicinity of the notopodial sac. Implantation in this region not only gives a plentiful blood supply but also removes any danger of injury, during the operation, to the nerve cord of the host.

In *Clymenella* the nerve cord lies outside of the circular muscles and is virtually a part of the integument (see fig. 14). In removing pieces of the cord, therefore, small amounts of ventral body wall about the cord were included. In all cases care was taken to keep this extra material at a minimum. One very important point to note, however, is that all pieces included some mesodermal elements which were very intimately associated with the nerve cord. It has been shown in a number of polychaetes that many neoblasts are present along the ventral nerve cord of an intact worm. Probst (1931) reported that neoblasts, present in this region of *Aricia,* produce practically all of the new tissues in posterior regeneration. Faulkner (1932) found a similar situation in *Chaetopterus.* Studies of *Clymenella* lead the author to believe that much the same condition also exists in this worm. It is clear then that the so-called nerve cord implants included at least a little hypodermis and some mesodermal elements including muscle tissue and probably many neoblasts.

For controls, pieces of body wall or wall of the digestive tract were used. The latter included, of course, both endodermal and mesodermal elements. The pieces of body wall probably included all of the general types of cells present in the nerve cord implants, with the exception of the neoblasts and the elements of the nerve cord itself.

Control Animals

Thirty worms served as hosts for control implants. Five of these implants were portions of the digestive tract, the others were taken from various parts of the body wall except in the vicinity of the nerve cord. Six of the hosts, including 3 with gut implants, died within a few days. Of the survivors, 13 lost the implants. The remaining 11, all with body wall implants, lived from 9 to 27 days. In none was there any evidence of bud formation. In all there seemed to be a certain amount of resorption of the implant. In those surviving 3 weeks or more a scar with possibly a minute pimple-like elevation was all which remained as external evidence of the operation.

Experimental Animals

A nerve cord implant was inserted in the dorso-lateral part of the thirteenth segment of each of 69 worms. Sixteen of these animals died within 10 days after the operation. In 30 other cases the implant either dropped out or slipped into the coelomic cavity. No buds formed on any of these.

Of the remaining 23 worms, in which the graft took, 6—including 2 which lived more than 30 days after the operation—showed no evidence

of new tissue at any time. Of the remaining 17, 3 produced irregular buds and 14 formed tails at the implant region. The development of these tail buds followed a quite consistent schedule. The first unquestionable evidence of new tissue was usually on the fourth or fifth day. By the seventh or eighth day a low anal collar with minute cirri had appeared, developing about the original dorsal side of the protruding portion of the implant in such a way that the external surface of the latter was exposed. Segmentation began to be evident on about the tenth day and was usually quite distinct, with notopodial setae well developed, two or three days later. The position of the notopodial setae indicated that the implant was in the mid-ventral part of the base of the bud.

Three of the tail buds consisted of ten segments, the proximal seven of which were setigerous. In the intact animal this is the number and organization of segments posterior to the implant point, if the thirteenth segment is included. Two of these buds (Figs. 1 and 2) did not differ appreciably in external appearance from the usual type produced in ordinary regeneration at this level. The implant came from the eleventh segment of the donor for the bud in Fig. 1 and from the second segment for the other bud. The third bud (Fig. 3) was curved toward the side in which the implant—taken from the second segment of the donor—was located. The anal collar was open on the same side. Evidently the amount of tissue produced along the ventral part of the bud was considerably less than in other parts. In ordinary posterior regeneration at the eighth to eleventh segments, buds with one side of the anal segment short or missing often occur (Sayles, 1934). Also, at the twelfth to fourteenth segments one side of the anal collar is usually shorter at first. In contrast with this implant bud, however, the open side is probably always dorsal in regenerates.

Four of the implant tail buds were of 9 segments. With the exception of a small part of the thirteenth segment, this is the number found in the intact worm posterior to the level of the implant. Two of these buds (Figs. 4 and 5) were of the ordinary tail type. The implants for these came from segments 10 and 8, respectively. Segment 8 also supplied the implant for another bud which was short, bent, and with a double anal segment (Fig. 6). The fourth bud of this group—implant from the sixth segment—terminated in an anal segment which was open on one side, only about one-half of the segment being present. From the last setigerous segment of this bud there arose a secondary part consisting of a slender stalk at the end of which was another anal segment, also open on one side. Unfortunately this worm died before a careful drawing had been made.

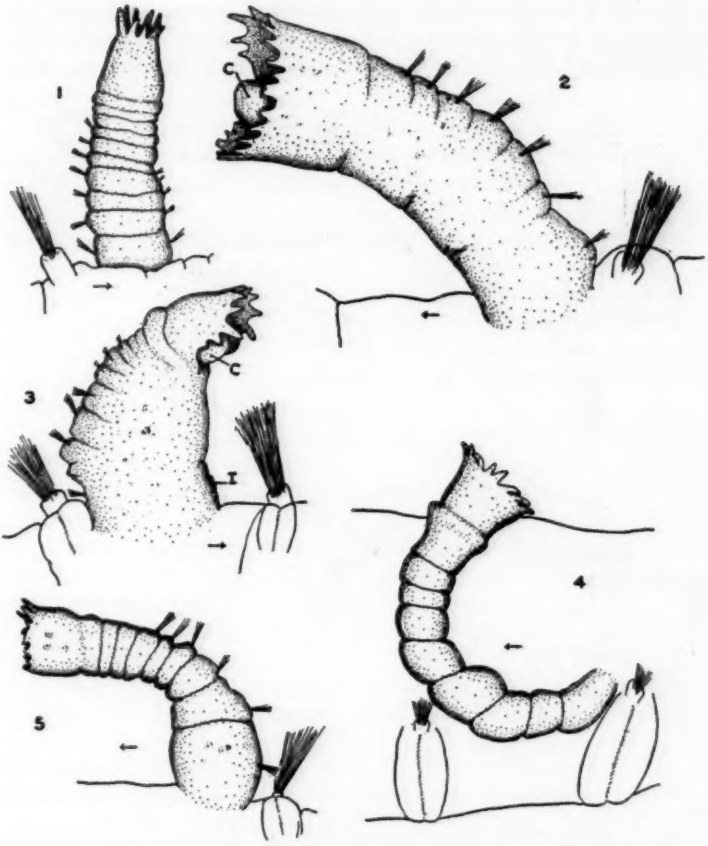

FIG. 1. Dorsal view of part of thirteenth segment of host bearing 10-segment induced tail bud. Age, 15 days. Arrow points toward anterior end of host in this and all other figures. Except where otherwise noted all figures are camera lucida drawings and × 22.

FIG. 2. Dorsal view of part of thirteenth segment of host bearing 10-segment induced tail bud. Age, 15 days. *C*, anal cone.

FIG. 3. Ventral view of part of thirteenth segment of host bearing 10-segment induced tail bud. Age, 19 days. *C*, anal cone; *I*, exposed part of implant.

FIG. 4. Left lateral view of thirteenth segment of host bearing 9-segment induced tail bud. Age, 26 days.

FIG. 5. Ventral view of part of thirteenth segment of host bearing 9-segment induced tail bud. Age, 16 days.

One bud (Fig. 7) consisted of 7 segments, 4 of these setigerous, and terminated in an anal collar of the usual type. From the ventral side of the middle of this bud there had developed a three-segment secondary

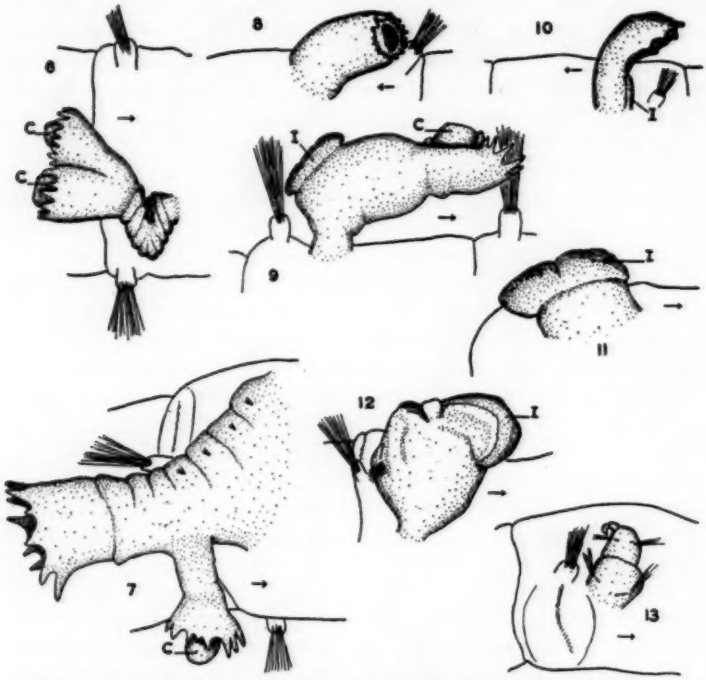

FIG. 6. Dorsal view of part of thirteenth segment of host bearing 9-segment induced tail bud with anal segment double. Age, 30 days. *C*, anal cone.

FIG. 7. Dorso-lateral view of part of thirteenth segment of host bearing induced double tail bud. Age, 18 days. *C*, anal cone.

FIG. 8. Dorsal view of part of thirteenth segment of host bearing induced tail bud. Age, 10 days.

FIG. 9. Ventral view of part of thirteenth segment of host bearing induced tail bud. Age, 14 days. *C*, lobulate anal cone region; *I*, exposed part of implant.

FIG. 10. Left lateral view of part of thirteenth segment of host bearing induced bud consisting almost entirely of anal segment open on one side. Age, 11 days. *I*, exposed part of implant. Enlarged from small, rough sketch. About × 20.

FIG. 11. Right lateral view of part of thirteenth segment of host with implant, *I*, raised up on small amount of new tissue. Age, 15 days.

FIG. 12. Right dorso-lateral view of part of thirteenth segment of host with implant, *I*, raised up on mass of new tissue. Age, 44 days.

FIG. 13. Right lateral view of part of thirteenth segment of host bearing induced bud consisting of two setigerous segments followed by a slender, twisted region. Age, 26 days.

tail of good shape except for the large size of the anal cone. Microscopical preparations of this bud show a division of the nerve cord of the main bud running into the extra part. In this case the implant came from the tenth segment.

Three of the tail buds showed little or no external evidence of segmentation. This condition was probably due in part to the fact that they lived only 10–14 days after the operation. Two of these were of the usual type with complete anal collars but with exceptionally large anal openings. One of the latter (Fig. 8) was the result of an implant from the sixth segment; the other, lost before an accurate drawing was made, arose from an implant from the twelfth segment. The third bud in this group (Fig. 9) was bent sharply toward the anterior end of the host. The implant—from the seventh segment—could be seen at the outside of the bend. On the same side of the bud the anal segment was open.

Each of the three remaining tail buds consisted of little more than an anal segment open on one side. In two of these the exposed part of the implant was proximal to the open portion of the bud. No record was made of the position of the implant in the third. The third, fifth, and seventh segments supplied the implants for these. In each of these cases an endeavor was made to keep the animal alive to observe further developments. As a result not one was preserved or carefully drawn. Figure 10 was prepared from a rough sketch of one of these, made 11 days after the operation.

Of the three irregular buds, two apparently consisted of the implant raised upon a small mass of new tissue. This new tissue showed no evidence of differentiation in one case (Fig. 11) but included a single notopodium in the other (Fig. 12). The third irregular bud (Fig. 13) was made up of two well-formed setigerous segments followed by a slender, hook-like distal region. This condition was probably not the result of the breaking off of the tip of a developing bud as the bud was slender when it was first forming on the eighth day and the end began to curve dorsally on the tenth day. The implants in these cases came from the eleventh, sixth and second segments, respectively.

Some of the internal features associated with these buds are well illustrated by the bud of Fig. 8. An oblique section through the base of this bud, showing its relation to both host and implant, is shown in Fig. 14. The implant forms a large mass lying in the coelomic cavity ventral to the digestive tract. The great thickness of the implant hypodermis, as contrasted with that of the host, is due in part to the fact that the implant is cut in an oblique plane but chiefly to the fact that it really was thicker. The body wall in the posterior part of *Clymenella* is thin,

somewhat transparent, and includes few glands. In the anterior region, on the other hand, there is a very thick, opaque and highly glandular integument. At the ninth segment there is a distinct line of demarcation between these two regions. This particular implant bud came from the anterior region (sixth segment).

Several points brought out by a study of serial sections of this animal apparently hold true of such other worms of this series as have been studied. The digestive tract of the bud is completely independent of that of the host. It is apparently formed by cells from the regeneration

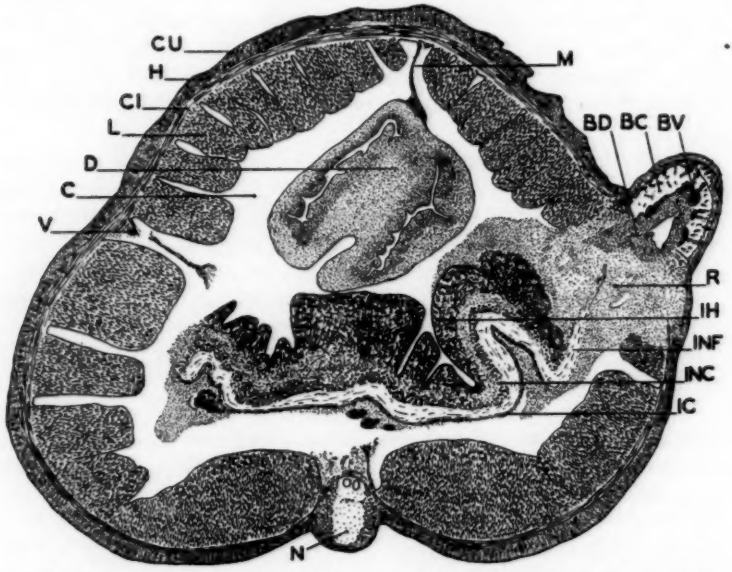

FIG. 14. Cross-section through host and oblique section through base of implant bud of Fig. 8. Age, 10 days. *BC*, coelom of bud; *BD*, digestive tract of bud; *BV*, blood vessels of bud; *C*, coelom; *CI*, circular muscle layer; *CU*, cuticle; *D*, digestive tract; *H*, hypodermis; *IC*, circular muscle tissue and regeneration cells of implant; *IH*, hypodermis of implant; *INC*, cell bodies of implant nerve cord; *INF*, fibers of implant nerve cord; *L*, longitudinal muscle; *M*, dorsal mesentery; *N*, nerve cord of host; *R*, mass of regeneration cells; *V*, lateral longitudinal blood vessel. × 40.

mass. There is also no evidence of any continuity between nerve cords of implant and host. The tip of the implant cord, attached to the right side of the body wall, is directed toward the ventral side of the bud. The ventral side of both bud and implant is toward the dorsal side of the host. Thus the bud is developing at the end of the implant with

the same orientation as one would have found in a bud which might have regenerated at the end of this same nerve cord if the latter had been left in place in the donor.

Discussion

The buds described above have apparently all been produced as the result of the implantation of a piece of appropriate tissue from an adult, non-regenerating animal. At the present time studies are being carried on in an endeavor to learn: (1) what cellular elements are contributed by host and by implant; (2) whether the elements of the implant important in inducing the bud are the neoblasts or the nerve cord or both. Some evidence has already been obtained to indicate that the neoblasts may be essential. This appearance of a bud in association with an implant cord is not in accordance with the ideas expressed by P. L. and G. H. Bailey (1938) who found that in *Eisenia* and *Nereis* organization of tissues into normal structures is dependent upon the presence of the nerve cord "in its normal relation to the other tissues."

Of the 17 cases in which new tissue was formed at the implant, 14 were tail buds and 3 were irregular. In no case was a bud formed which in any way resembled a head, even of the modified type found in anterior regeneration at certain levels (Sayles, 1936). From the point of view of regeneration, the 22-segment body of *Clymenella* may be divided into three regions, namely: (1) segments 1–7, levels at which regeneration occurs in the anterior direction only; (2) segments 8–11, a transition zone in which lost posterior ends are more and more accurately replaced the more posterior the level and in which the reverse is true of lost anterior ends; (3) segments 12–22, levels at which anterior regeneration does not occur but lost posterior portions are completely replaced. Of the 14 implants associated with induced tail buds, 8 were from the anterior region of the donor, 5 were from the middle region, and 1 was from segment 12. (For the irregular buds 2 implants were from the anterior zone and one from the middle one.) Since the implant was put into the thirteenth segment of the host in each case, all were in the posterior, tail-forming zone. These data point clearly to the fact that at the thirteenth segment, the host and not the implant determines the nature of the bud to be formed. In 7 cases the great influence of the host is further indicated by the presence of buds of nine or ten segments, while nine and a fraction segments exist posterior to the implant. This is all the more impressive when we note that two of the 10-segment buds resulted from implants taken from the second segment, a level at which only a single head segment may ordinarily regenerate. These results are in accord with those of Baskin (1933) and Crowell (1937).

The implant does, however, seem to control the orientation of the new bud. In each bud which developed at the exposed end of an implant the dorsal and ventral parts bore the same relation to the implant as one would expect to find between nerve cord and bud in ordinary regeneration. For example, in the case of the bud shown in Fig. 3 the implant was placed with its ventral surface toward the anterior end of the host. When the bud developed its ventral side was toward the anterior end of the host and the implant showed in the proximal part of this side.

A tentative explanation may be offered for the condition found in two of the irregular buds (Figs. 11 and 12). In each of these cases the implant was relatively short and healed into the wound region throughout most of its length. As in other cases the new tissue developed on the original internal, or dorsal, side of the implant. In these two worms this new tissue was, therefore, beneath the implants and raised them up from the body wall of the host. Under these conditions the new tissue formed was apparently very limited in amount and in differentiation.

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THE EFFECTS OF CENTRIFUGING ON THE POLAR SPINDLES OF THE EGG OF CHAETOPTERUS AND CUMINGIA

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The factors involved in the movement of the maturation spindle to the pole of the egg are obscure and have been little commented on. In many eggs it has been shown that the mitotic rays may extend through almost the entire polar hemisphere. In such eggs the axis of the spindle is described as at first horizontal, or possibly somewhat oblique. It then turns with one end directed to the pole, but whether there is one particular end that takes this position or whether either end may so orient is unknown. It is conceivable, if there is a predetermined end, that it may trace back to the last oogonial division, or if not, that a cytoplasmic difference may determine the direction of its turning. At present there is no evidence for either of these assumptions.

In some respects the eggs of *Chaetopterus* offer excellent material for the study of the conditions that lead to the movement of the meiotic spindle to the pole. The eggs can be procured in any quantity, and the same individual will supply material for two or more weeks. When removed from the parapodia the germinal vesicle is present in the eggs. It breaks down within a few minutes and the polar spindle forms, which moves within ten minutes to the pole of the egg where it remains until the egg is fertilized. The first polar body is given off 20 to 22 minutes after the egg is fertilized, and the second polar body about ten minutes later. Since all the eggs are in the same stage an opportunity is given to study the effects of centrifuging on the spindle at any stage desired.

If the egg is centrifuged at the time when the wall of the germinal vesicle is disappearing, and centrifuging is continued during the time when the polar spindle is being formed deep in the egg, the spindle may be prevented from passing to the pole or to any other point on the surface. Whether it does or not may depend in part on the centrifugal force employed; in part on the orientation of the egg during centrifuging; in part on the destruction of the peripheral rays of the mitotic figure; and in part on the redistribution of the contents of the egg.

If the egg is centrifuged after the polar spindle has reached the pole

its position there or its change of position will depend on the orientation of the egg with respect to the centrifugal force. If the egg is centrifuged during the time of extrusion of the polar body the result may again depend on its position with respect to the centrifugal force and, theoretically, at least, on whether the spindle is in anaphase or telophase or whether the second polar spindle is in process of formation.

Again, if the egg is centrifuged after the first polar body has been given off, the second spindle may remain in place or be driven into the interior. If the latter occurs the question arises whether it will return to the surface and give off the second polar body, or remain in the interior of the egg. These alternatives may depend on the location of the pole and also on whether the egg is elongated by the centrifugal force, or remains spherical or nearly so.

The first problem then is to find out whether the eggs do or do not orient on the machine with the polar hemisphere turned toward the center of rotation. In *Chaetopterus* the orientation of the eggs on the machine has been discussed by Whitaker and Morgan (1930), Wilson (1930), and Morgan (1937*b*, 1938). It has been found that there is a tendency for most of the eggs to orient more or less with the polar hemisphere toward the center of rotation, but in a considerable number of cases the pole may make any angle with the centrifugal axis up to as much as ninety degrees or even more. I have suggested (1938) that the failure of many eggs to orient perfectly may be due to the contents of the eggs beginning to respond to the centrifugal force more quickly than the egg as a whole. The best evidence for this is found when the eggs are centrifuged after the first polar body has been extruded. Under these circumstances the polar body frequently does not lie at the centripetal pole, but to one side of it. In such cases there can be no question of a change in the position of the true pole. If the eggs are strongly centrifuged before the spindle has reached the pole there is the possibility that the presence of the oil cap may interfere with the extrusion of the polar body in the oil field. This possibility has also been examined. If the centrifugal force is not too strong the oil field extends as a broad thin cap over the centripetal pole, and the spindle may pass through its center, or in most of the eggs at one side. In other eggs the spindle lies outside the cap. On the other hand, if the centrifuging is strong, or over a longer time, then the oil cap is smaller in circumference and extends deeper into the egg. If, as seems to be the case, fewer polar bodies are given off in its center, this is in part due to the small arc it covers, but possibly the oil may then interfere with the passage of the spindle through it. In such cases the spindle may pass to the surface outside the oil cap, which may mean that it does not come

off exactly at the true pole but near it. There is no serious objection to such an assumption, but it is difficult to prove because other evidence shows that the egg fails in many cases to orient with the pole exactly in the axis of rotation.

The way in which the stratification of the materials of the egg takes place has been little discussed by those who have used the centrifuge to separate the materials of the egg. If it is assumed that at first the oil, the clear cytoplasm, the yolk and the pigment are equally distributed in the egg, they may be considered as passing by or through each other according to their specific weights. On the other hand, these differences may be of such a kind that one of them, the oil for instance, may move more quickly to one end than does the yolk or the pigment to the opposite end, while the clear protoplasm comes to occupy the middle zone. Determinations of the so-called absolute viscosity of the egg, by measurements of the time it takes to bring about a visible stratification of these materials, are open to the criticism that some of the materials reach their positions sooner than do others. For example, the yolk and the pigment of the egg of *Cumingia* may take a longer time to be separated clearly than does the oil and its underlying clear zone. If the clear zone is the matrix from which the other materials are separated, then, I suppose, it is this matrix that is responsible for the viscosity, but I am not sure that this is the correct interpretation. However, in most cases reported, comparisons are made between eggs in different stages of maturation, and such comparisons may be more nearly valid.

When the ripe ovarian eggs, or eggs just set free, are killed in Flemming and later sectioned and stained there is found a ring of black granules in the protoplasm around the nucleus. The nucleoplasm remains unstained in iron haematoxylin. After the germinal vesicle breaks down this region stains blue. If the ovarian eggs are killed in aceto-carmine, sectioned, and stained in iron haematoxylin fewer dark granules are seen. Whether there are two kinds of granules, or, in the latter case, fewer are stained is uncertain.

If the egg is on the centrifuge when the germinal vesicle breaks down the larger granules move along the periphery towards the centrifugal end forming there a semicircle in section (Fig. 39). They are imbedded in a somewhat homogeneous layer that stains yellow in eosin which is thickest around the antipole outside the granules. In the center of the outer hemisphere there is a clear spherical area, sometimes having a flocculent appearance slightly stained by eosin. In it are a few dark granules. This clearer sphere does not appear to be due to failure of the preservatives to penetrate into it. Its origin is obscure. It looks as though it were the clear nucleoplasm, but that part of the egg is now

occupied by a finely granular stained zone (as it is also in the uncentrifuged egg). Over this clear sphere there is a crescent of very finely granular substance that stains very dark in iron haematoxylin (Fig. 40). The chromosomes are sometimes imbedded in this crescent or lie just outside it.

The eggs of some other species of animals have a more protoplasmic layer around and beneath the pole, while the yolk lies mainly in the antipolar hemisphere. If such eggs are centrifuged, and orient on the machine, the oil present in the clear region or in the yolk must pass through the protoplasmic region while the yolk simply becomes more condensed in the opposite hemisphere, leaving the clear zone nearly in its original place. But if the egg is not so oriented at the time when the redistribution begins there is an alternative possibility. The protoplasmic layer may move around one side of the egg, and the yolk around the opposite side. The polar spindle, if not attached to the surface, may then be carried along with the protoplasmic layer. When removed from the centrifuge, the stretched protoplasm may move partly back to the original polar field, and the polar body be given off at or near the pole. I have shown this to be the case in the egg of *Ilyanassa*, and there is some evidence that such a slipping around may take place in the centrifuged egg of the frog, even in response to gravity in an inverted egg. In *Crepidula* a similar movement may take place although Conklin's figures do not give any evidence of such a rotation.

In *Ilyanassa* the rotation of the interior of the egg was best demonstrated either by holding the egg in an inverted position in a small tube or by imbedding inverted eggs in gelatin (dissolved in sea water) that was solidified before placing on the machine (Morgan, 1935). In the latter experiment the question may arise whether the gelatin itself affects the result. The same question has arisen in the course of the present experiments when eggs were centrifuged on top of a gum arabic solution, or in or above an "isotonic solution" of cane sugar or raffinose. In fact, these methods were abandoned after the first experiments because it was found that some of the solutions not being quite isotonic caused the egg to shrink slightly and possibly inhibited, for a time, the movement of the spindle, or interfered with its movements on the centrifuge. Even when the eggs come to lie on top of a gelatin or gum arabic solution they lie in a gradient where the sea water mixes with the gum arabic, etc. This effect will be considered later. It suffices here to say that the stratification of the eggs can be as perfectly brought about when they lie on the gum as in sea water alone, but in most cases these eggs do not elongate or break apart as in sea water, and if kept too long in the solution the extrusion of the first or second polar body may be suppressed.

Centrifuging the Egg during the Dissolution of the Nuclear Membrane

By means of the centrifuge it is possible to prevent the polar spindle from moving to the pole; or later, after it has moved to the pole, to carry it back into the interior of the egg. Under these conditions there are two alternatives. The chromosomes may then separate, but whether the mitotic figure will be capable of dividing the egg into equal or unequal cells, or whether the double or quadruple set of chromosomes remains in place and takes part later in the cleavage division can only be determined in each kind of egg by examining it. The other possibility is that during the first or the second polar division one end of the spindle, attached to the surface, may remain in place and the other end be carried deeper into the egg (as Conklin has described for the egg of *Crepidula*), and bring about a division sometimes into two large cells. In *Chaetopterus,* and also in *Cumingia,* I have not succeeded in doing this—the spindle and its attached chromosome either remaining at the surface after centrifuging, or moving as a whole deeper into the egg. Different eggs probably behave differently in this respect. In *Ilyanassa* the polar spindle in " inverted eggs " is carried along in the protoplasm at or near the surface of the egg while in *Crepidula* the spindle is driven through the center of the egg or at least it appears to do so judging by Conklin's drawings, although he does not specifically state this to be the case. This kind of result may depend to what extent the eggs orient on the machine; for, if some or many of them lie obliquely, when centrifuging begins it is to be expected that the lighter and heavier parts may slip around each other rather than interpenetrate.

Both in *Ilyanassa* and in *Crepidula* a spermatozoön has entered the egg while in the oviduct, hence the polar spindle is activated. On the other hand, *Chaetopterus* does not give off its polar bodies unless the egg is fertilized. Such eggs must be fertilized in order to activate the mitotic division. The egg can be activated by the sperm either before it is centrifuged, when the conditions are more nearly comparable to the eggs of the other animals, or it can be first centrifuged and then fertilized. Both tests have been made. It is also possible to break apart the eggs on the centrifuge and obtain large or small tops. It might seem that the likelihood of division by means of the polar spindle would be thus facilitated, but this is found not to be the case. Even very small tops may give off small polar bodies.

When many eggs are centrifuged in sea water they make a compact mass at the bottom. In squirting them loose, with a long large-mouthed pipette, some will, no doubt, be broken apart if they have become elongated and have a narrow neck, but it is surprising how many even

of the latter remain intact. Unless the membrane is broken the eggs do not greatly elongate, but if the centrifugal force is strong enough the membrane breaks at the yolk region and the yolk squeezes out leaving the rest of the egg surrounded by the shriveled membrane. Sometimes more, sometimes less, of the egg escapes, and it may be pinched apart at almost any level. The oil, too, after heavy centrifuging, may pinch off, and either float freely or remain inside the membrane.

In order to avoid breaking the eggs apart and still elongate them for an examination of the subsequent behavior of the polar spindle, they were sometimes centrifuged over a sugar solution heavier than sea water or over gum arabic. This method avoids packing the eggs together at the end of the tube as they do in sea water. The packing can be avoided by adding only enough eggs over the sugar or gum to form a single layer on the surface. It was found, however, that the sugar, and the gum also that was used, bring about some shrinkage of the egg and that the movements of the spindle may be retarded or even stopped. This method had, therefore, to be abandoned since in many of the experiments it was desired to move the spindle and not interfere with its activity, for, if it is held back, along with the rest of the egg, it will, when the eggs are returned to sea water (where they quickly become more or less spherical), proceed under most conditions either to come to the surface and produce polar bodies or else skip that stage if held as long as an hour on the centrifuge, hence defeating the purpose of the experiment.

Formation of Polar Spindle

Since the condition of the egg at the time of centrifuging had a bearing on the results, it became necessary to prepare a normal series of stages for comparison. When the egg of *Chaetopterus* is removed a large nucleus is present in the center of the egg that has an irregular contour. In the living egg its outlines disappear in a few minutes and in the course of 11 minutes a clear oblong area appears at the pole. Stained preparations show that the chromosomes have not yet reached the pole at the time when the clear area is first formed. They lie near the center in a cluster or clump for 9 minutes. Nine tetrad chromosomes are present, one smaller than the others. At 12 minutes they lie in a spindle that is moving to the surface.

Centrifuging the Eggs in Sea Water

Several different experiments were made in which the time of centrifuging and the force applied to eggs in different stages of polar body formation were tested in order to find out whether the polar spindle, if

driven into the egg, would cause the egg or an egg-fragment to divide by means of the polar spindle, or return to the surface.

In some of the experiments the unfertilized eggs were centrifuged at the time when the chromosomes had just emerged from the germinal vesicle. The chromosomes are then near the center of the egg in the polar hemisphere and it might be supposed that they could be held there during the time that it takes for the spindle to form and move to the pole. This is in fact what sometimes happens. If then the eggs are removed from the centrifuge and fertilized an opportunity might seem to be given for the buried spindle to divide the egg, but in no case did this happen. The chromosomes either remained in the interior and took part in the later division of the egg, or, under some conditions, moved to the surface and gave off one (usually) or two polar bodies before the cleavage came on.

The alternative, viz., to fertilize the eggs at the time when the polar spindle has formed and then centrifuge them, was also tried. Under these conditions it seemed possible that the activated spindle might divide the egg while on the centrifuge, or later, if taken off at the proper intervals; but again the results were negative. The spindle moved to the surface and gave off one or two polar bodies, or remained in the egg and the chromosomes took part later in the cleavage.

Several attempts were made to drive the polar spindle (after fertilization) into the interior at the time when it was about to divide to produce the first polar body but, as a rule, the spindle and its attached chromosomes either remained at or near the surface, or if driven into the interior, it failed to divide the egg at the time of polar body formation. Since, in previous experiments with *Ilyanassa,* it had been found, though very rarely, that the second polar spindle may divide the egg (if it is elongated at that time), a number of experiments were made with this stage but without success.

In nearly all cases stained preparations were made of the eggs in order to check up the records of the living eggs. These confirmed the observations on the living eggs, but added very little further information except with respect to the location of the chromosomes.

It is obvious, then, if the eggs fall, to some extent, at random on the centrifuge, that the spindle (and chromosomes) would have to move across the diameter of the egg or through its center or along one side in all cases where the pole of the egg happened to be at the centrifugal pole, since the chromosomes come to lie in the protoplasmic zone of the centrifuged egg, or near it, or in the oil field. On the other hand, in the reverse situation the spindle (and chromosomes) would, if it moved at all, shift to the interior. In all intermediate positions the chromo-

somes would already lie at or near the level toward which their specific gravity tends to locate them. It has been shown in previous papers that the egg of *Chaetopterus* on the centrifuge tends to orient on the machine with the true pole more or less turned towards the center of rotation. Hence, in the majority of cases, there would be little tendency to shift the level of the spindle, that had already reached the surface, to a new position. In fact, this corresponds with the observations on preserved eggs centrifuged at that time. Practically no spindles were found at the apex of the oil but not infrequently at its border. Also none were ever found in the yolk that occupies about half of the egg. If a spindle happened to be in what becomes the yolk hemisphere after centrifuging (in an egg completely inverted), it would be driven into the lighter half of the egg.

Though it has not been possible to induce the polar spindles to act as a division center for the whole egg, or even for a part of an egg, the result is not due to a destructive action on the spindle, since it may after centrifuging move to the surface and give off one or two polar bodies. The failure is also not due to the egg not being elongated at the time when the spindle division is due, since very many cases of elongated eggs were observed. It may be said then that the failure of the polar spindle to divide the egg of *Chaetopterus* is due in part to the tenacity with which the spindle adheres to the surface after it has once reached the surface (except as explained above), or, in other cases, to the strong tendency for the spindle to come to the surface. But granted that these conditions occur in most of the cases, it still remains unexplained why the polar spindle, if held inside the egg during the time of its normal formation, is unable to divide the egg, or a fragment of the egg. In this connection, it should be recalled that the division of the egg of *Crepidula* or of *Ilyanassa* to form the so-called giant polar body is a relatively rare event.

Details of Centrifuging

(1) The eggs were fertilized 2 minutes after removal, and 5 minutes later were centrifuged at 2080 r.p.m. at 19½ cm. from center for one-half hour. When removed (Figs. 1, 2) a group of chromosomes was present either in the center of the stained zone or at one side. The eggs were not much elongated. No polar bodies had yet been given off. Later the eggs rounded up and a few showed polar bodies. One hour and 5 minutes after fertilizing about half of the eggs had divided. *Comment:* The polar spindle had not reached the surface (after 7 minutes) when the eggs were centrifuged. They were kept on the machine for a half hour during which time the first polar body was overdue. The stained preparations show that the chromosomes had come to the surface on the machine, but the polar body was not extruded. It is probable that the formation of the polar spindle had been interfered with. Even after removal few polar bodies were found.

(2) Three minutes after removal the eggs were fertilized and kept for 13 minutes. They were then centrifuged for 5 minutes at 1110 r.p.m.; for 5 minutes

at 2080 r.p.m.; and for 5 minutes at 2750 r.p.m. The eggs were somewhat elongated (Fig. 3). No polar bodies had been given off. A group of chromosomes surrounded by a clear area was present in the stained zone. The center of the yolk was more translucent than the periphery. Some of the eggs gave off one or two polar bodies 20 minutes after the normal eggs. Later many eggs divided, most with one, a few with two polar bodies; but in some eggs no polar bodies were found. *Comment:* The polar spindle must have reached the surface (after 16 minutes) before the eggs were centrifuged. After 15 minutes on the centrifuge no polar bodies had been given off although overdue about 10 minutes. Later some eggs showed a polar body 10 minutes after those of the normal control. It appears that the polar spindle had retreated or had been driven into the interior, but later, in some of them at least, it came to the surface and gave off one or two polar bodies when returned to sea water.

(3) Five minutes after removal eggs were fertilized and 11½ minutes later were centrifuged at 2080 r.p.m. for 5 minutes; then for 5 minutes at 2750 r.p.m. When removed the eggs were somewhat elongated (Fig. 4). The chromosomes are at the surface of the stained zone. A spindle is not evident. The centrifuging (after 16½ minutes) was too late or not strong enough to dislodge the chromosomes from the surface. The eggs showed polar bodies (one, or two?) 12 minutes after removal. The eggs had become more rounded. Antipolar lobes came in soon after this (Figs. 5, 6, 7). *Comment:* These eggs, in preparations, showed usually only one polar body, or none at all. The lobes in most eggs were in the yolk region, but it is noticeable that in many eggs the antipolar lobe came partly in the stained zone (Figs. 5, 6, 7), which means that such eggs had not oriented on the machine. Half of the eggs (those in the opposite tube) were kept on the centrifuge (at 2750 r.p.m.) for 7 minutes longer. They are more elongated, and the chromosomes are still attached to the surface (Fig. 8). Eggs of this lot were preserved 17 minutes after removal when some of them were dividing. The dividing eggs have one polar body; those not yet divided showed none in nearly all cases, although the large asters for the first division were present. *Comment:* The eggs were centrifuged about 4 minutes before the first polar body was due. They were on the machine for only 10 minutes. Polar bodies came off 10 minutes after removal, generally only one. Eggs of the same lot were centrifuged for 7 minutes more. They were more elongated and the chromosomes were at the surface. One polar body came off, but whether this corresponds to the first or the second normal polar body can not be stated. In either case the number of chromosomes after fertilization should be three-fold.

(4) After standing for 5 minutes the eggs were fertilized and then, after standing for 10 minutes more, were centrifuged at 1700 r.p.m. for 30 minutes. The eggs were somewhat elongated (Figs. 9, 10), but no polar bodies had been given off, at least from most eggs. A clear nucleus was found in some eggs (Fig. 9) and a bunch of chromosomes in others (Fig. 10). A few began to divide 5 minutes later. In some eggs, where the constriction for the first furrow is appearing at the top of the stained area, the oil is pinched off as a separate globule (Figs. 11, 12, 13, 14), and if it happens to contain the polar body nucleus *on the surface* it resembles a large polar body. But there can be no doubt that this is an event taking place long after the polar division took place. The eggs in the opposite tube were centrifuged for 8 minutes longer (Figs. 15, 16). They were more elongated; some gave off a polar body in or near the oil, others did not. A nuclear vesicle, or a group of chromosomes, lies in the stained zone. *Comment:* These eggs were centrifuged about 5 minutes before the polar body was due and kept on the machine for 30 minutes longer. No polar bodies were present, at least in most eggs, and a nucleus or a collection of chromosomes was present in the interior. Evidently the chromosomes had retreated from the pole, but the polar spindle did not divide the egg while on the machine.

(5) After 7 minutes in sea water the eggs were fertilized, and stood for 10

minutes in sea water when they were centrifuged at 1110 r.p.m. for 40 minutes. When removed the eggs were elongated and, some at least, had given off a polar body (or two) (Figs. 17, 18). They began to divide 10 minutes after removal (Fig. 19). *Comment:* These eggs were centrifuged about 3 minutes before the polar body was due. Some eggs gave off a polar body while on the machine. They divided 10 minutes after removal (Fig. 20), without polar bodies or sometimes with only one.

(6) Eggs, after standing 5 minutes, were fertilized and stood for 10 minutes. They were centrifuged at 2080 r.p.m. for 25 minutes and for 15 minutes at 2750 r.p.m. They were much drawn out and broken; many middles were present. Eggs in one of the tubes were centrifuged for 12 minutes more. These too were much drawn out and many broken. The cylindrical eggs contracted and 12 minutes later some of the whole eggs showed the oil pinched off (Fig. 21). Some of the middle also showed a constriction (Fig. 22). This was 10 minutes at least before the cleavage was due. Without an examination of stained preparations the large clear globules containing oil might seem to be giant polar bodies, but preparations show they are without nuclei and quite clearly due to partial fusion of top and middle. *Comment:* The eggs were centrifuged 5 minutes before the polar body was due, and at a higher speed than the preceding for 40 minutes. One or two polar bodies had been given off, and after removal a few eggs showed the oil region constricted off, but since these bodies did not contain a nucleus and often had one or two polar bodies at the surface, it is clear that these bodies are due to the protoplasm pinching in two, and not due to a division by the polar spindle.

(7) Eggs were kept in sea water for 3 minutes, then fertilized and after 5 minutes were centrifuged at 1110 r.p.m. for 3 minutes; at 2080 r.p.m. for 2 minutes; and at 2080 r.p.m. for 15 minutes. The eggs were elongated (Figs. 23, 24). The chromosomes were at the surface in the stained area, generally in the more faintly stained neck, sometimes in two groups. Rarely a polar body was found, though, as a rule, no polar bodies had been given off. The eggs were contracted 8 minutes afterwards (Figs. 25, 26), but no polar bodies were present. Later some of the eggs divided without polar bodies; other eggs had not divided at this time. *Comment:* The eggs were centrifuged 8 minutes after removal, hence about 12 minutes before the polar body was due and probably before the spindle had reached the surface. They were on the centrifuge for 20 minutes. The polar body was overdue when removed. The preparations show the polar chromosomes at the surface but only rarely a polar body had been given off.

(8) Eggs stood 5 minutes in sea water and were then fertilized and after 8 minutes more were centrifuged at 2080 r.p.m. for 10 minutes. The eggs were oval. Preparations show a group of chromosomes in the center of the egg in the stained zone. No polar bodies were observed in the living egg, or in the preparations. Most of these eggs cleaved at the same time as the normal. In the opposite tube the rest of the eggs were centrifuged at 2750 r.p.m. for 10 minutes longer. These were more elongated. The chromosomes were still in the center of the stained zone (rarely at the surface). No polar bodies came off, and no giant polar divisions were observed. *Comment:* The eggs were centrifuged 13 minutes before the polar body was due. The spindle must have been at or near the surface when the eggs were put into the machine. Centrifuged for only 10 minutes the eggs were not much elongated. The chromosomes were found in the preparations in the interior of the stained zone. No polar bodies came off after removal and the eggs cleaved at the normal time. Other eggs of the same lot, centrifuged 10 minutes longer, gave the same results.

(9) The next set was not fertilized. After standing 6 minutes the eggs were centrifuged for 10 minutes at 1110 r.p.m.; then for 10 minutes at 2750 r.p.m. (Figs. 27, 28). The eggs were, for the most part, separated into middle and bottom; the oil was off in most cases. In the middles the chromosomes were found at the surface (in clumps) (Figs. 27, 28). Some middles were drawn out

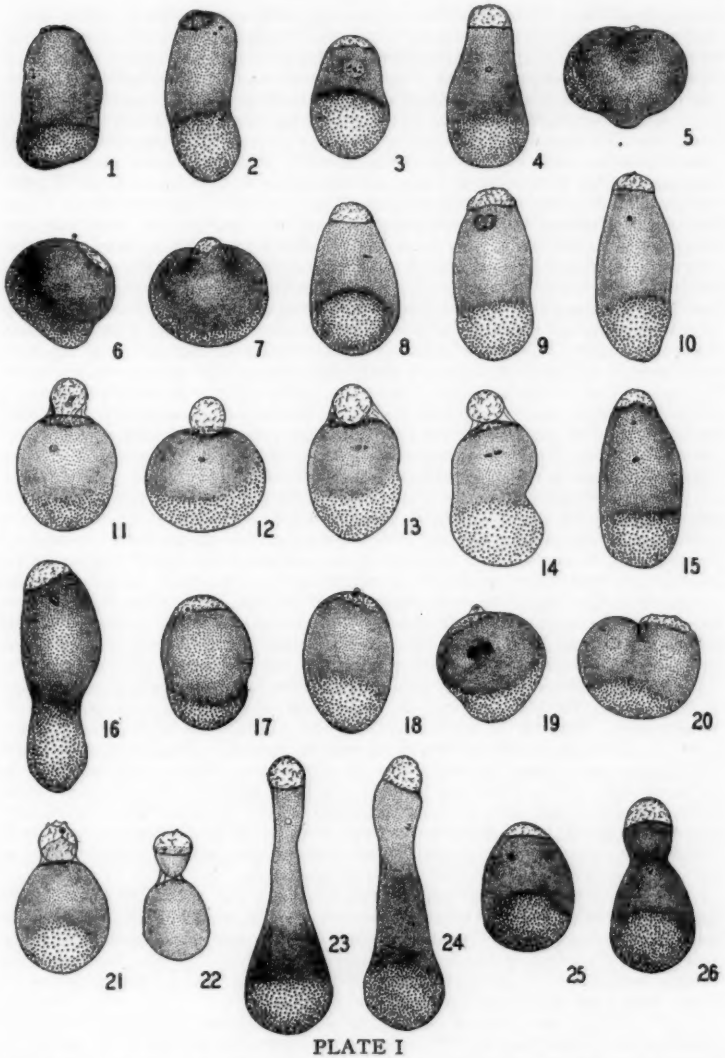

PLATE I

into long strands. In the opposite tube the eggs were taken off after 20 minutes. They were elongated and fewer were broken apart (Figs. 29, 30, 31). They were then fertilized at 9.51, i.e. after two minutes. Seven minutes later they were round. Eleven minutes later a polar body was not present in most eggs. The eggs began to divide after 23 minutes (Figs. 32, 33, 34, 35) but did not show any polar

bodies as a rule. *Comment:* This set of eggs was not fertilized before centrifuging. The eggs were put in the centrifuge 6 minutes after removal. At the time the germinal vesicle was just breaking down. They were centrifuged for 20 minutes. Later, when fertilized, they did not give off a polar body, or perhaps rarely. The meiotic spindle did not bring about division of the eggs.

(10) Unfertilized eggs stood in sea water four minutes; they were then centrifuged for 15 minutes at 2080 r.p.m. The eggs were much elongated. Fertilized, they contracted, some into two parts (as shown in Fig. 36). Later, some of them had a polar body at the side, and later still divided with an antipolar lobe. Eggs in the opposite tube were centrifuged at the same rate for 10 minutes longer. They were more elongated, and many consisted of two bulbs connected by a narrow neck; others were broken apart. The eggs were fertilized, and in the course of 10 minutes most of them had contracted into an oval form; but a number of them contracted into two spheres joined by a narrow neck. Preparations of these showed that the two parts were not the result of a division, as shown by the position of the chromosomes in the outer sphere and the presence of a polar body at the surface (Figs. 36, 37, 38). *Comment:* This experiment shows that even when the eggs were elongated at the time of fertilization the meiotic spindle did not divide the egg. If by chance it had happened to lie in the neck, it is possible that conditions there might have led to the separation of the anaphase plates, but if this should happen the division of the protoplasm into two parts would not be due to the meiotic figure but rather to the contraction of the distal and proximal parts, or bulbs of protoplasm and the breaking of the connecting strand.

(11) Unfertilized eggs were put into the centrifuge tubes one minute after removal when the germinal vesicle was still intact, and rotated for 5 minutes at 1110 r.p.m. The contents had begun to be stratified and the germinal vesicles had disappeared. After killing in picro-acetic, the sections were stained in iron haematoxylin and saffranin. A broad oil cap is present, followed by a clear zone (stippled in Fig. 39). The centrifugal end of the egg has a clear faintly coagulated reddish sphere. A deeply-stained, blue crescent of finely granular material lies over it. Centrifugal to this clear area is a half circle of large, deeply-stained granules. The outermost layer is stained red and is without obvious granules. The scattered chromosomes lie in the clear zone, about the middle of the egg, and just above the blue crescent. A large black nucleolus lies somewhere in the same region. Beneath the egg-membrane there is a layer of minute red granules. I do not know what material makes up the blue crescent, perhaps chondriosomes. The deeply-stained black granules may be called yolk, which leaves unnamed the red outer layer of the distal end in which the black granules are imbedded. Some of the remaining eggs were fertilized 5 minutes later. The extrusion of polar body was little delayed, if at all, in these eggs. It appeared most often at the edge of the oil field or even in its center. Later most of the eggs divided, but most of them showed only one polar body in the preparations.

Half of the same lot of eggs were centrifuged for 5 minutes more at 1110 r.p.m. (Fig. 40). They gave nearly the same picture as before (Fig. 39), except that the chromosomes are collecting around an aster, and the nucleolus has disappeared. The oil area is smaller and, after fertilization, the polar body area lies outside the oil field. The extrusion of the polar body was delayed (8 minutes). Later some eggs were found to have two polar bodies.

(12) Unfertilized eggs of another lot were put at once in the centrifuge and rotated for one-half hour at 2080 r.p.m. Many eggs were broken. The preserved, intact eggs, sectioned and stained in Delafield's haematoxylin, showed four distinct layers with the chromosomes arranged around a clear body, probably the nucleolus, in the center of the inner stained zone, or at its surface. No mitotic spindle was found in either case. The rest of the eggs were centrifuged one-half hour longer. The conditions were practically the same as in the preceding set. In both cases some eggs were later fertilized. One or two polar bodies came off, and later most tops and whole eggs divided.

It will be noticed that when the eggs are centrifuged at once the germinal vesicle breaks down, setting free the chromosomes in the middle layer of the egg. Remaining on the centrifuge for one-half to one hour the group of chromosomes either stays in place, or is carried or moves to the surface. The spindle must develop later in some eggs, at least, if they are fertilized, since a polar body may be given off. In other cases the chromosomes remain in the interior, possibly because the egg itself has passed to a later stage of its development, or because the meiotic spindle does not develop, or because if it does, it fails to carry the chromosomes to the surface. Also to be taken into account is the fact that the mass of eggs is crowded together at the end of the centrifuge tube where a lack of oxygen may slow down the development. In such cases the whole egg would be expected to be held back, and then resume its processes at the stage where it was stopped.

(13) The eggs were allowed to stand for 15 minutes in sea water. The polar spindle would then be at or near the surface. The eggs were centrifuged for 15 minutes at 2080 r.p.m. Most of them were killed in Flemming, sectioned, and stained in haematoxylin and eosin. As shown in Fig. 41 five zones are easily recognized in the somewhat elongated egg. The oil cap is deep; beneath it lies a stained granular zone. The spindle lies in this zone near the surface. Next comes a less stained zone of finely granular material. This is followed by a zone of larger granules that run in streaks as though moving outward. Then follows a zone of large granules or lumps buried in a less stained material (probably the red stained material of eggs killed in picro-acetic stained with eosin).

The other half of the same lot were centrifuged 15 minutes more at 2080 r.p.m. The eggs were more elongated (Fig. 42) and showed the same five layers as those of Fig. 41. The distal dark layer is more nearly fused with the outer black layer, but the preparation is so black that the distinction between these two layers is more difficult to see. Later, the centrifuged eggs of this set were fertilized after 40 minutes. Some eggs showed polar bodies at one side (after 15 minutes), then became pear-shaped; a lobe appeared and the eggs divided normally at the normal time. Neither the elongation nor the stratification interfered with normal cleavage.

A Double Egg

One giant egg was found in a series of sections of centrifuged eggs (Fig. 43). It is here drawn to the same scale as the others, but owing to imbedding etc., it is more shrunken. Its double size is shown by comparison with other normal-sized eggs (Fig. 44) on the same slide. The normal eggs show polar spindles in anaphase with 9 chromosomes in each half-plate (Fig. 45). The chromosomes of the giant are scattered in the center of what appears to be a large bipolar spindle (Fig. 46). There are about 18 chromosomes, which is double the normal number, but if they have already divided this would not be the case. There is no real evidence, however, that they have divided as yet, and moved apart.

Fused Eggs

Fused eggs were found in two preparations (Figs. 47–51). The eggs had stood for 3 minutes in sea water, were then fertilized, and after 13 minutes were centrifuged at 1110 r.p.m. for 2 minutes, at 2080 r.p.m. for 5 minutes and at 2750 r.p.m. for 15 minutes. The eggs were

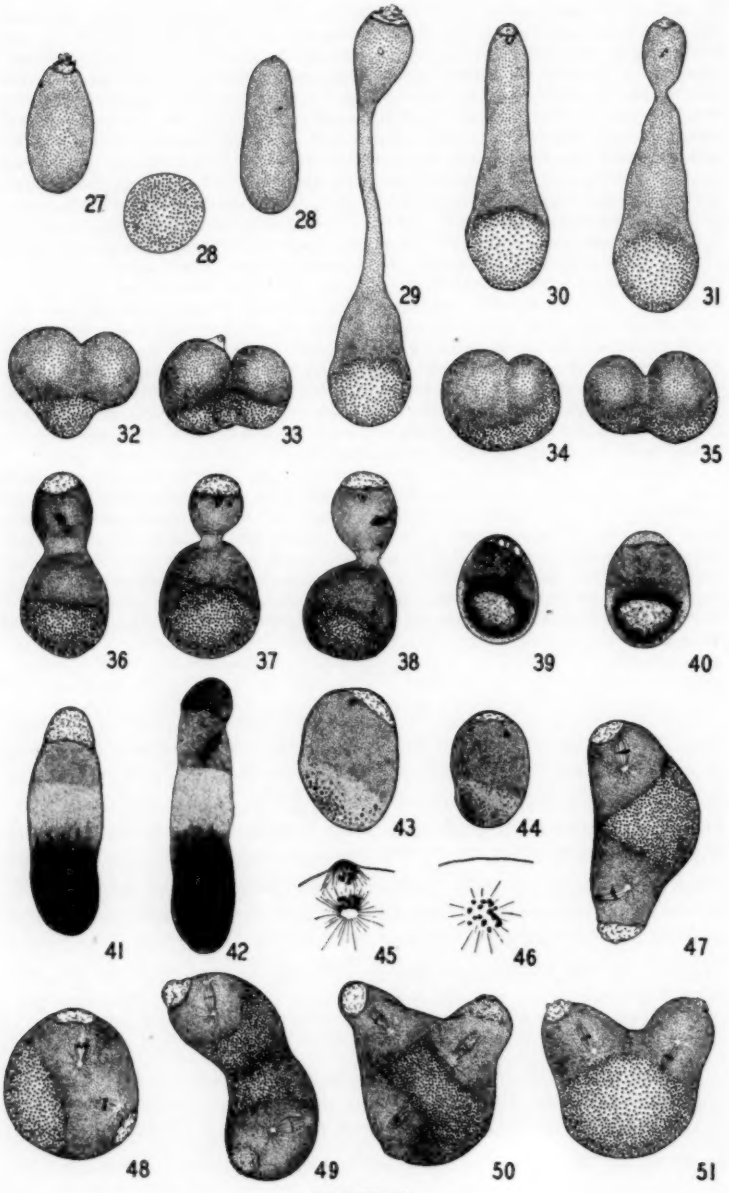
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PLATE II

about twice as long as wide. Amongst them were three "fused eggs" (Fig. 50). The yolks are completely fused but the protoplasmic parts are still separated and each contains a polar spindle. These eggs were killed 28 minutes after fertilization. There is one other case in the same preparation in which five eggs are fused, at least they have a large common yolk, the protoplasm and oil of each making different angles with the yolk. The fused eggs must have been stratified before fusing, since the stained zone and oil cap make different angles with the yolk.

There is one other preparation (Fig. 51) that has one double egg. It had been treated in much the same way as the last, but its history is not entirely clear.

Centrifuging the Eggs of Cumingia

When the eggs of *Cumingia* are ejected from the siphon there is present a well-developed spindle that lies somewhat excentrically in the interior of the egg in a more or less horizontal position or else pointed toward the pole. It remains there until the egg is fertilized and then moves to the pole. When the unfertilized egg is centrifuged the spindle remains in the clear zone (see below). After removal to sea water and fertilization, the polar bodies are given off without respect to the stratification, which means that the eggs are little, if at all, oriented in the tubes. The eggs are faintly pink; some lots have more pigment than others. Most of the pigment lies in a broad zone in the outer region of the egg. When centrifuged the oil is quickly driven to the centripetal end. When centrifuged for a longer time (one hour) at a higher speed, the pigment is driven to the end opposite the oil leaving a broad clear middle zone that fills most of the egg. If this zone is carefully examined it is found to consist of a clear zone beneath the oil and a faintly yellowish zone next to the pigment. There is a rather sharp boundary line between these two if the eggs have been sufficiently centrifuged. If the living egg is stained, by adding a little methyl green to the sea water, the zone next to the pigment becomes slightly bluish-green, increasing the difference between it and the protoplasmic zone.

In 1908, 1909, and 1910 I described the results of centrifuging this egg. Four zones were recognized, but the difference between the protoplasmic and yolk zone was not clearly distinguished and what is identified as yolk in the sections (1910, Plate II, Fig. *N–U*) is the pigment plus probably some yolk also. In 1934 Costello recognized four zones identifying the yolk and the pigment as two separate zones. Stronger centrifugation than I had employed was used by Costello, *viz.* 6000 gravity.

Sections of eggs killed immediately show a small clear vesicle just

beneath the surface not previously recognized. Its origin and nature was not determined, but in a set of eggs that had stood three-quarters of an hour in sea water there was observed a clear cap on the eggs surrounded by a faint ring of pigment. Whether this cap is derived from the clear vesicle is not known. This cap had been seen in 1910 and recognized, but such eggs may have stood for some time in sea water.

Sections were made of normal eggs and of those after centrifuging. They were killed in Flemming or in Bouin solutions and stained in haematoxylin and different dyes. The normal egg, after Flemming, contains a number of deeply-stained granules that are scattered through the protoplasm around the spindle, except at one point, the pole of the egg towards which the spindle is pointed. When the living egg is centrifuged these granules are driven to the centrifugal pole where they fill a segment of the egg that is red or pink in color. This region stains yellow after certain killing and staining reagents, and the granules in it are difficult to see and perhaps tend to fuse together. In the earlier paper (1910, Plate II) this region is spoken of as the yolk. Sometimes black stained pigment granules lie more or less scattered in this yellowish "yolk" depending on methods of preparation. Whether there is present in addition to the pigment granules another material in this part of the centrifuged egg in which the dark granules are imbedded is uncertain. The region next to this is distinguished from the clear protoplasmic region only with difficulty in the living centrifuged egg. In sections of stained eggs it can also be distinguished from the protoplasm zone. Whether it is "yolk" material in the ordinary sense, or another kind of substance, or yolk of a somewhat different nature, remains uncertain.

When the living centrifuged eggs are put into Flemming solution after centrifuging the pigment segment immediately becomes brown. The oil segment at the opposite end more slowly becomes brown, but later jet black. It holds this color throughout the treatment with the reagents subsequently used (water, alcohol, xylol, etc.), but the pigment section loses its dark stain and may stain a light yellow if picric acid is present or stains red in eosin. In some of my earlier papers (1910) the pigment region of the centrifugal hemisphere of the living egg is colored red. These eggs were not centrifuged as long or at as great a speed as were those mentioned above, where the pigment is driven further into the centrifugal pole to form there a red segment. On the other hand, in some of the stained sections (Plate II) there is a yellow segment that is spoken of as yolk and no dark granules are indicated. As stated above, this "yolk" is either the pigment granules that have

coalesced in the preserving reagent, or a substance in which they are imbedded.

Drawings of other sections of eggs (in that paper) that were probably preserved in another reagent (Flemming), show the pigment granules scattered in the protoplasm before centrifuging; and after centrifuging they are represented, as here, driven to the centrifugal region.

The presence of a large mitotic spindle in the interior of the *Cumingia* egg suggested that the egg might be a suitable object to examine whether the spindle could be induced to divide the egg, producing a "giant polar body." However, since this spindle and its equatorially arranged chromosomes do not become active until a spermatozoön enters the egg, it becomes necessary to fertilize the egg either before or after centrifuging to activate the spindle. Both procedures have been used, but with two possible exceptions no such division was brought about.

Eggs centrifuged before fertilization, removed to sea water and then fertilized give off at least one, and in most cases probably both polar bodies. Eggs centrifuged after being fertilized give off polar bodies either on the machine or after removal. The length of time in the centrifuge tube was also taken into account, since eggs that were not lying in the axis of centrifuging or even inverted would be expected to behave differently if the centrifugal force was sufficient to keep the polar spindle from moving to the true pole. If, on the other hand, the pole should lie at the more centripetal end of the protoplasmic zone, the movement of the spindle and its surrounding rays might even be facilitated while on the centrifuge. In the earlier experiments that I made (1910) on the egg of *Cumingia* I found that the polar spindle may push its way through the yolk or pigment or oil and give off the polar bodies at the surface. This means that neither of these regions presents a serious obstacle to the movements of the spindle, although statistical evidence is lacking as to whether to some extent there may be some difficulties in reaching the pole when the yolk underlies it. Moreover, in these cases the centrifuging was low, and not long enough to separate visibly the pigment and yolk fields. If centrifuged at a higher rate, or for a longer time, the outcome might have been different.

A few examples may be cited as follows: Unfertilized eggs were centrifuged for 15 minutes at 1664 r.p.m. at 10½ cm. from the center of rotation. When removed the eggs were not elongated or very little so. Fertilized after 5 minutes many eggs were seen to be giving off a polar body after 15 minutes at the side of the clear zone. No giant polar bodies were formed. A few eggs about twice the size of others were recorded in this set.

Another set was fertilized and stood for 12 minutes in sea water and

was then centrifuged for 10 minutes at 1664 r.p.m. They were spherical or columnar, and a few showed a dent at the side. After 6 minutes some eggs extruded a polar body. Only a few eggs divided; also the controls were not normal.

One set centrifuged as above was then fertilized. Eight minutes later polar bodies were observed, some of them exceptionally large. No second polar body was observed 7 minutes later, but 5 minutes later one polar body was seen in some eggs and rarely two. A deep constriction was observed in some eggs at this time. The eggs divided 10 minutes later.

The following cases apply to eggs centrifuged after fertilization. Since the polar spindle is active only after fertilization it is here rather than in the preceding cases that we might hope to bring about a division of the egg by means of the polar spindle, especially when the eggs were kept on the centrifuge during the active phase of the spindle.

Four minutes after fertilization the eggs were centrifuged for 20 minutes at 1664 r.p.m. The first polar body is expected about 20 minutes after fertilization. Two minutes after removal an egg was found divided into two nearly equal parts. This is one of the two cases observed that might be interpreted as due to the polar spindle dividing the egg. Nine minutes later polar bodies were observed in some eggs. Eleven minutes later the eggs divided. In the control, normal eggs, the first polar body came off 21 minutes after fertilization. There was, then, some delay in polar body extrusion in the centrifuged eggs.

Another set stood for 10 minutes after being fertilized. They were then centrifuged for 12 minutes at 3260 r.p.m. When removed the first polar body had been given off while in the centrifuge tube in some eggs. Later, when the eggs were divided, one, two or no polar bodies were found.

Another set was fertilized and allowed to stand until the first polar body was out (18½ minutes). This was an attempt to find out whether the second polar spindle was more efficacious in dividing the egg than the first one. The eggs were centrifuged for 28 minutes at 1664 r.p.m. They were then a little oval, and some showed a slight constriction. No divisions were present.

These few experiments are only exploratory. The negative results, with two possible exceptions, can not be understood to exclude the possibility that under other conditions the polar spindles would divide the egg, but the experiments were not promising.

Morgan and Tyler, in 1935, centrifuged the eggs of *Urechis* under conditions similar to those described here. In one respect the egg is different from those of *Chaetopterus* and *Cumingia;* the germinal vesicle

remains intact until the eggs are fertilized; it then breaks down, and the dent in the egg disappears. Eggs centrifuged before fertilization give off the first polar body at the true pole and produce normal embryos. If centrifuged after fertilization the chromosomes are driven towards the pigment side of the egg (centrifugally), and the polar bodies are given off somewhere in that hemisphere; if they are given off at all. The evidence indicates that these eggs fall at random on the machine, hence in many cases the polar bodies are given off at other regions of the egg than that of the true pole, as Conklin also found in *Crepidula*. The evidence also shows that the first and second cleavage planes pass through the secondary pole, but these eggs do not produce normal embryos. It can not be said, therefore, that a new polarity has been imposed on the egg, in so far as "polarity" is an essential feature of development.

When the eggs are on the centrifuge at the time when the chromosomes are set free from the germinal vesicle and the polar spindle is in process of formation, separate asters may appear in the same regions as the chromosomes. Later a spindle may develop between two asters and these are the eggs that give off polar bodies. When, on the other hand, the chromosomes remain in the egg, most of them are collected on the cleavage spindle; but some may remain outside attached to the astral rays. The abnormal development of the embryo may, in part, be due to the irregular distribution of the chromosomes.

If eggs are centrifuged after the extrusion of the first polar body, the second polar spindle may be displaced from the pole, but does not bring about the division of the egg. Whether it returns to the pole or gives off a polar body at some other point was not definitely settled, although in a few cases two polar bodies were observed at different points on the surface. In none of these experiments were the eggs kept long enough on the centrifuge to pass by the time when the polar bodies are formed in the normal egg. In this respect the experiments with *Chaetopterus* and *Cumingia* cover somewhat different grounds.

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THE REPRODUCTIVE CYCLE OF THE VIVIPAROUS TELEOST, NEOTOCA BILINEATA, A MEMBER OF THE FAMILY GOODEIDAE[1]

I. The Breeding Cycle

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Introduction

Much attention has been devoted recently to the reproductive cycles of viviparous teleosts. Some of the recent work has been concerned with the histological phases of the gonad during reproduction (Scott, 1928; Bailey, 1933; and Turner, 1933, 1938*b*) but more attention has been devoted to different aspects of the breeding cycle. In this respect, much of the work has been concerned with members of the family Poeciliidae (order Cyprinodontes) because of the large number of viviparous species available and because of their relative abundance (see Turner, 1937*a* for recent work on the poeciliids). With the exception of three papers (Turner, 1933, 1937*b*; Mendoza, 1937) nothing more has been reported on the family Goodeidae from the central plateau of Mexico, a family which contains twenty-six viviparous species. In view of the dearth of information on the goodeids, this investigation was undertaken to study the female reproductive cycle of *Neotoca bilineata* in order to throw some light on the reproductive activities of this viviparous family. The information obtained in this investigation involves three phases of the reproductive activity of *Neotoca:* (1) the breeding cycle, (2) the germ cell cycle during gestation, and (3) the somatic cycle of the ovary. The present paper is devoted entirely to the phenomena associated with breeding as observed in the laboratory: the breeding season, the lengths of the brood intervals, the sizes of broods, superfetation, fertilization, and color variations in the female. The periodic variation of the germ cells in the ovary and the gross and microscopic changes which the ovarian soma undergoes during gestation will be dealt with in later papers.

[1] I wish to express my appreciation for the advice and guidance of Dr. C. L. Turner under whose direction this investigation was undertaken.

Materials

This form was chosen because of the number of females available, their convenient size, and because of the rapidity of brood production. Specimens of *Neotoca* used during the investigation were raised in the laboratory. A record of over eighty females was kept during the course of the investigation; approximately thirty-five of these were killed and serial sections made of the ovary for microscopic examination. No single female was kept during the entire period of observation, which lasted from the fall of 1934 to the spring of 1937, since specimens frequently died or were killed during critical periods of the year and were replaced by new females. The individual females under observation always were isolated with one or two males, the isolation tanks consisting chiefly of one- or three-gallon aquaria. All tanks were filled with seasoned water and abundantly stocked with plants. With the exception of a few aquaria, all were kept in the same room, subject to a fairly constant room temperature of about 70° F. and the normal, daily fluctuation of light. Electric lights were turned on but infrequently at night so that any possible effects of artificial illumination on the reproductive cycle were negligible. Records were kept of the date of isolation of the females, frequency of brood production, number and size of young, and the condition of the maternal gonad at time of death. In most cases, when females were killed a unilateral dissection of the ovary was made and measurements of the young on that side were taken; the opposite side of the ovary was left intact for sectioning. In a few females, the entire ovary was sectioned without the unilateral dissection.

Breeding Season

The following description of the breeding season is based solely on laboratory observations; there has been no study of the females in their natural habitat such as was performed by Scott on *Fitzroyia lineata* (1928) and by Turner on *Brachyrhaphis episcopi* (1938*a*).

Table I shows the preponderance of broods appearing during spring and early summer. Series A consists of older females isolated during the early fall of 1934. Of the nineteen females isolated, only six had October broods and only one of the six started further gestation that fall. The other five specimens already had started a period of sexual inactivity or rest destined to last until the following spring. The remaining females of this series were allowed to live through the fall and winter but they produced no more broods; nevertheless, the following spring all but one of the females started to breed again. Evidence of a period of

reproductive inactivity over the winter is obtained from the fact that over half of the females in Series A had no fall broods. Additional evidence of this winter inactivity appeared constantly during the investigation.

Early in 1935, the individual females of Series B were isolated and records of brood production were started. It is evident from Table I that but few broods were produced during January and February; however, almost all females produced broods during March and April. During May there was a decrease in the number of broods followed by a further decrease during June and July. The latter month marks the termination of brood production. Females that were alive after July bore no young during the summer, fall or winter.

The females of Series C were isolated during the fall of 1935. With the exception of one specimen which had young during December, no other broods were produced until March. It is evident again that broods appeared predominantly during spring and early summer. July again marks the termination of brood production.

Series D comprises a small group of females isolated during the fall of 1936. Of about one dozen females isolated, only three had had young by February of 1937 at which time the records were discontinued.

For some reason not understood, many deaths were recorded during the months of May, June, and July whereas deaths during other times of the year were most infrequent. The marked mortality evident in the females so jeopardized the regularly breeding specimens that frequently they were sacrificed during July and preserved rather than to risk death and subsequent loss of the tissues for histological examination. If it had not been desired to get a complete histological record of the ovary during gestation, females would have been permitted to live as long as possible so as to get a continuous record of breeding throughout various seasons. It is possible then that despite the deaths of some females, few would have produced a small number of broods during summer, fall, and winter. The total number of broods produced during each month over a period of three years was as follows:

Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
6	10	19	29	18	14	10	0	0	4	2	1

In summary it may be said that brood production occurs chiefly during spring and early summer whereas the greatest number of broods appears during the month of April. The months of August and September show no brood production. This may be caused in part by the fact that most females had been killed or had died by the latter part of July. Among the females that remained, however, no brood production

was observed. It is very likely that, had older females been used, more fall broods would have been recorded.

Brood Intervals

Some viviparous fishes such as *Poecilistes pleurospilus* and *Heterandria formosa* (Turner, 1937*a*) are characterized by a marked decrease

Table I

The complete record of breeding from the fall of 1934 to the early spring of 1937. The number of the different females is merely for purposes of identification. The columns marked "Brood I," "Brood II," etc., indicate the dates of the first, second broods etc.; the dates are expressed as fractions, the numerator indicates the month of the year, the denominator indicates the day. The columns marked "Interval" include the interval of days between successive broods. Days are recorded on which the females were killed or died naturally; the asterisk following the date of death indicates that the female was bearing young at the time.

Female		Brood I	Interval	Brood II	Interval	Brood III	Interval	Brood IV	Female killed	Female died	
	1	10/1							11/12*		
	2	10/8							11/12		
	3	10/11							11/15		
	4	10/22							11/19		1934
	5	11/7							11/21		
	6	11/14							11/21		
Series A	8								2/6*		
	9								2/6*		
	10								2/6*		
	11								2/6*		
	12								2/6*		1935
	13								2/6*		
	14								2/6*		
	15								2/23		
	16	2/15							3/4*		
	19	3/9								4/1	
	1	1/8	62	3/11						5/7	
	2	1/29	50	3/20	15	4/4				5/10	
	3	2/2	44	3/17	40	4/26	39	6/4		7/4	
	4	1/1									
	5	5/29									
	6	2/10	71	4/21	48	6/8				7/9	
	7	2/13	55	4/8					4/26		
Series	9	2/15	45	3/31	41	5/11					1935
B	10	2/15	17	3/3						5/17	
	11	3/4	43	4/16	41	5/27					
	17	3/11	45							5/4	
	18	3/10	66	5/16						6/3	
	19	3/21	40	4/30	40	6/9	40	7/17	7/29*		
	20	3/16	41	4/26	44	6/9	40	7/19	7/24		
	21	3/23	36	4/28	44	6/11				7/17	
	22	3/23	42	5/4	36	6/10	40	7/20	8/5*		
	23	3/27	43	5/9	48	6/26			7/21*		

TABLE I (*continued*)

Female		Brood I	Interval	Brood II	Interval	Brood III	Interval	Brood IV	Female killed	Female died	
	24	3/27	51	5/17	54	7/10			7/21		
	25	4/1	41	5/11	44	6/24					
	26	4/19							7/20		
	27	4/5	42	5/17	44	7/1			7/21*		
	28	4/6								6/4	
	29	4/6	31	5/7							
	30	4/9	48	5/27							
	31	4/9								7/12	
	32	4/9	45	5/24							
Series	33	4/16								6/4	1935
B	34	4/15	46	6/1						7/15	
	35	4/20									
	36	4/25	40	6/4	45	7/19				7/20	
	37	4/24								5/21	
	38	4/26									
	40	5/28									
	43	5/21									
	44	5/13									
	46	6/3	47	7/20							
	47	7/19							8/11		
	48	3/23	60	5/23						7/8	
	49	12/1	120	4/29	64	7/2				7/8	
	56	4/23	49	6/11					6/19*		
	57	3/1	55	4/25							
Series	59	6/1	39	7/9					7/16		
C	60	3/23	43	5/6							1936
	63	5/6									
	66	6/20							6/20		
	67	3/7									
	72	4/8	49	5/27						7/16	
	73	3/1	41	4/17					6/14*		
Series	76	1/15									
D	77	1/23									1937
	81	1/12									

in length of brood intervals with the advance of the breeding season. Intervals between broods decrease from as much as twenty-six to twelve days in the former and from forty-one to three or four days in the latter. In *Neotoca,* however, as the breeding season advances there is no apparent increase in the rapidity of brood production. Furthermore, the average length of the brood intervals occurring during different months of the breeding season show no significant difference in length throughout the season (Table II).

Most brood intervals are regular and fluctuate around a normal fixed period. Sixty-eight per cent of the 51 recorded intervals show a maximum variation of 9 days (39–48 days) whereas fully 51 per cent of the intervals vary but 5 days (40–45 days). The numerical average for

TABLE II

Average length of brood intervals throughout the breeding season.

Time Occurrence of Intervals	Average Length of Intervals
	days
February–March	43.6
March–April	43.8
April–May	47.3
May–June	43.4
June–July	45.8

the entire group is 45.0 days. Although most intervals are fairly regular, a few variations ranging from 15 to 71 days were recorded. As an example of regularity in brood production, the following case is cited (Table III). It involves the parallel breeding behavior of four females (Series B) occupying adjacent tanks.

TABLE III

The breeding record of four females in adjacent tanks.

Female	Brood I	Int.*	Brood II	Int.	Brood III	Int.	Brood IV
19	March 21	40	April 30	40	June 9	40	July 19
20	March 16	41	April 26	44	June 9	40	July 19
21	March 23	36	April 28	44	June 11		
22	March 23	42	May 4	36	June 10	40	July 20

* Interval in days.

In addition to the above example of parallel breeding, several other instances led the writer to suspect that breeding was unusually plentiful on certain days, as if there were regular waves of brood production among several females. Analysis of the brood record resulted in the following interesting conclusions. There occurred three distinct groups of females, with members of each group having parallel breeding cycles. The breeding period for each group normally lasted about ten days and appeared again approximately forty-five days later. The number of females in each group varied from four to eight individuals, each female producing two or more broods. Each group retained its identity despite the fact that members within a group did not produce their broods in any regular order. The groups furthermore retained the same relative

order in their breeding periods throughout the season: *ABC, ABC, ABC, AB, A.* The groups were not composed entirely of females starting brood production during the same month; some females started breeding later in the season and yet their cycle corresponded with one of the group cycles already established. Of a total of 80 broods produced during 1935 between January 1 and July 21, 58 fit into this regular cyclic phenomenon; 13 broods could not be accounted for in this scheme since they were first and only broods; finally, only five females producing a total of 9 broods participated in irregular cycles which failed to fit into the above rhythmic activity. The breeding records other than those of 1935 were too scanty to permit such an analysis.

It is true that the selection of groups was made arbitrarily. There is probably nothing that determines rigidly the number of days during which members of a group will produce young, but segregation of the females was made possible because, regardless of the order of brood

TABLE IV

Increase in size of broods during the breeding season.

Brood	Total number of broods	Total number of young	Average number of young per brood
First.....	53	302	6.0
Second.....	30	240	8.0
Third.....	15	163	10.8
Fourth.....	4	52	10.4

production within the units, the breeding period of one group did not overlap with that of another group. Regularity was maintained remarkably well during the season for a phenomenon which frequently is considered to be sporadic. Finally, the determination of these groups is not significant in itself; rather, the importance lies in that they demonstrate markedly the regularity of the breeding behavior of the females of this species. It is true, obviously, that there are minor differences in the rate of breeding among the individual females.

SIZE OF BROODS

The present analysis of the size of broods is based on records obtained from 53 females which produced a total of 103 broods. On the basis of the information available, it is evident that the broods increase in size during the season. This increase is apparent in the general averages of all first, second, third, and fourth broods (Table IV).

It is emphasized that the above evidence concerns in the main part young females producing their first broods; consequently, the increase in the average number of young per brood for successive broods actually implies that with growth of the females, broods become larger. Although the third and fourth broods were not as numerous as would have been desired, together they indicate the tendency for more numerous young in the third and fourth broods. The difference between the last two broods obviously is too small to be significant. It is to be expected that older females would have produced somewhat larger broods. Only a maximum of four broods is recorded for any one female. It is likely that females have more than four broods during their active reproductive life, but it is doubtful if they have more than four or five broods during one season since the interval between broods is so long.

Superfetation

An outstanding characteristic of the reproductive cycle of *Neotoca* is the absence of superfetation. This absence is concluded from three sources: the brood interval, the evacuation of young, and examination of the ovary itself. Broods are spaced far apart and are regular in their appearance. Furthermore, there is no evidence that normally only a part of the young are evacuated at one time. Generally, young are known to be born within minutes of each other. In only two females was there evidence of a phenomenon resembling superfetation. In both cases the first brood was small and was followed in two weeks by another small brood. Since it is improbable that the young were completely developed in that short a time, it is more likely that they were part of the first brood but for some unknown reason were not expelled along with the others. Since this phenomenon failed to appear elsewhere no significance was attached to its occurrence. Examination of many ovaries, both by gross and microscopic methods, failed to reveal the presence of more than one brood in the ovary at one time. Normally, all embryos in an ovary were in the same stage of development. It is true that slight irregularities in size occurred but they were seldom great. Therefore, it may be concluded that each brood is distinct in *Neotoca* because of the brood interval, the simultaneous evacuation of all young, and because examination of the ovaries in a large number of females revealed but one brood in the ovary at one time.

Fertilization

In viviparous fishes producing annual broods, copulation and fertilization may occur at the beginning of the breeding season or, as occurs

in *Cymatogaster aggregatus* (Eigenmann, 1892), copulation may take place several months before the breeding season, in which case the sperm are stored in the ovary until time for fertilization. Among viviparous forms producing several broods during one season, the time of copulation may vary. In forms such as the poeciliids, there is a continual introduction of spermatozoa by the male. However, storage of sperm has been reported for *Xiphophorus helleri* (van Oordt, 1928; Bailey, 1933) and has been determined experimentally in *Heterandria formosa* by Turner (unpublished data). In such cases, spermatozoa are stored and are capable of fertilizing several sets of ovocytes over a considerable period of time. Normally, however, it appears that the storage is not necessary because of the continual introduction of fresh spermatozoa by the male. There is no evidence that such a storage phenomenon occurs in *Neotoca;* in this species, copulation and fertilization occur concurrently or nearly so. Spermatophores which have been reported for some viviparous teleosts likewise do not occur in this species. The actual time during which fertilization occurs has been determined as follows. Examination of 7 ovaries with young in late stages of gestation failed to reveal spermatozoa in the ovary or any indication of fertilization. Three females killed at intervals of 5, 6 and 7 days after the birth of young likewise failed to reveal signs of fertilization. However, 5 females killed at intervals of from 7 to 15 days after the birth of young revealed new embryos in different stages of segmentation. Spermatozoa were evident in almost all of these ovaries. Although it is possible, of course, that fertilization occasionally may occur at a different time, it is concluded from the above evidence that normal fertilization occurs about the seventh day after the release of a previous brood. The details of the cytology of fertilization will be considered in another paper.

Female Coloration

Peculiar coloration of the females during certain phases of the breeding cycle has been reported by Scott for *Fitzroyia.* Although the regularity and the significance of these color changes have been questioned by some investigators, reference is made herein to a peculiar change in the color of the females. During the first year it was noticed frequently that the abdominal region of the female became a vivid blue whereas normally it is a pale gray. Furthermore, it was noticed that females with parallel breeding cycles assumed this coloration approximately at the same time. The coloration normally lasted a few days and then disappeared. If the female became excited, the color could disappear in a few seconds to return later in a gradual manner. Weekly observations

during the second breeding season indicated that in non-breeding females, the coloration appeared very irregularly whereas among the breeding females some semblance of regularity was observed. In most cases, the color appeared on an average of 10 days after the birth of a previous brood and 33 days before the birth of the following brood. It is significant that two virgin females, isolated from the time of birth, showed the same coloration simultaneously, at frequencies comparable to the cycles of breeding females. Microscopic examination of the virgin gonads, fixed during the color phase of the female, revealed the ovarian soma in a very active, turgid state similar to that of an ovary during early gestation. Thus it may be that this color is correlated in some way to a periodic activation of the ovarian soma, whether or not the female is exposed to males. Furthermore, the time of appearance of the color in the breeding females correlates closely with the time of fertilization, thereby suggesting that there occurs periodically in the ovary an inherent activation usually accompanied by color changes in the female and evidently facilitating copulation and fertilization.

Discussion

Compared with the breeding activities of other viviparous teleosts, *Neotoca* most closely resembles the poeciliids which in general breed actively during the spring and summer and but little during fall and winter. This seasonal wave of reproductive activity occurs in several genera including *Mollienisia, Xiphophorus, Gambusia* and others. These poeciliids and *Neotoca* obviously stand in contrast to viviparous forms such as *Cymatogaster, Fitzroyia* and others which have but an annual brood. As has been suggested by other investigators (Kuntz, 1913; Turner, 1937*a*), the writer also is of the opinion that the recurrent broods during the season are comparable to several annual broods rather than to one extended annual brood, as suggested by Barney and Anson (1921) for *Gambusia.* The former opinion is shared by the writer since in *Neotoca* there is a definite and complete activation of the ovary including separate copulation and fertilization for every brood.

Whether the laboratory breeding cycle is similar to that of the females in their natural habitat is unknown. The only information available is that obtained at the time the original stock was collected during March by Dr. C. L. Turner. Reproductive activity was present but not at a maximum. It has been shown for *Gambusia affinis,* at least, that the breeding records of the females in the laboratory and in the field correlate closely. On the other hand, Turner (1938*a*) has found that *Brachyrhaphis episcopi,* a poeciliid, fails to show this alternate high and low level of reproductive activity but breeds continuously throughout

the year in its normal habitat in the Panama Canal Zone. It is more likely that of the two, *Neotoca* resembles *Gambusia* more closely since the temperature and light factors are not quite as constant in its habitat on the Mexican plateau as is true for *Brachyrhaphis*. It is probable that the information on the breeding cycle of this species will apply to most goodeids although there is one exception known, *Goodea luitpoldi*, a form which is larger, matures more slowly, has greater brood intervals, and larger young.

The cause of the high mortality evident among the females during early summer is unknown. It is unlikely that death normally follows the production of one or two broods as has been reported for another viviparous form, *Comephorus baicalensis* (Dybowski, 1902). It seems more reasonable to assume that the females are at a low physiological level because of their reproductive activities and hence they are more susceptible to minor changes in food, temperature, or perhaps even to minute organisms in the water. Of these, temperature may prove to be the most important.

It has been shown that the breeding cycle remains quite constant throughout the season, differing from forms such as *Poecilistes* and *Heterandria*. It is indeed difficult to explain the cause of such a regular production of broods. It is not likely that external conditions are of major importance in the establishment of the regular cycles in *Neotoca*. However, it may be that food and temperature especially help to bring about the birth of young on some particular day or interval of days if the young are ready or nearly ready for birth. The regular cycles of brood production very likely are governed by some internal, inherent mechanism, a mechanism which is inherited in the species as a whole and probably expresses itself through hormonal control. Once brood production starts, the inherent periodicity expresses itself usually in a regular manner. However, in the absence of careful experimentation, it is unwise to discuss further the relative importance of external or internal factors in the determination of such a marked regularity in breeding activity.

Since the embryos remain in the ovarian lumen until time of birth, the ovary of *Neotoca* may be compared to the uterus of mammals. There is further similarity in that marked periodic activation of the ovarian soma occurs not only in breeding females but also in virgins. A third parallel exists in that fertilization evidently occurs during this period of activation. Consequently, in view of the above comparisons, it is suggested that there occurs in the female of this species a period similar to that of oestrus in the mammalian female.

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THE INFLUENCE OF TEMPERATURE ON THE SURVIVAL, GROWTH AND RESPIRATION OF CALANUS FINMARCHICUS

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Experiments which have been in progress at the Woods Hole Oceanographic Institution on the nutrition of the ecologically important copepod, *Calanus finmarchicus,* in relation to the food cycle of the sea have shed light on the types of food suitable for this species and on the rate of feeding of which it is capable under different conditions (Clarke and Gellis, 1935; Fuller and Clarke, 1936; and Fuller, 1937). Since the temperatures to which *Calanus* is subjected off our coasts vary greatly with the season and with the locality, it was desired to extend the investigation of the survival and growth of this species in the laboratory to include a larger range of temperature than had been possible previously. In the earlier experiments survival of the copepods had generally been poor, and Gross (1937) has expressed the opinion that even short intervals of temperature change, such as are entailed by the removal of the culture dishes from the constant temperature bath for inspection, are seriously detrimental to animals in culture. In the experiments about to be described we therefore proposed not only to test the effect of a variety of temperatures on growth but also to compare the survival of animals kept continuously at constant temperatures with that of other individuals subjected to periodic changes in temperature.

Calanus was shown to exist in the waters off Woods Hole throughout the year 1935–36 and to undergo reproduction during the spring months (Clarke and Zinn, 1937). Yet food organisms were not sufficiently abundant in these waters to meet the nutritive requirements which we have assumed for *Calanus* even at the maximum rate of feeding observed in the laboratory, if diatoms alone are considered (Fuller and Clarke, 1936; and Fuller, 1937). Food in the form of diatoms appeared to exist in only one-tenth the necessary concentration. This discrepancy has led to such suggestions as the considerable use of the nannoplankton by the copepods and this possibility should be investigated further, but the difficulty could be removed equally well if the

[1] Contribution No. 214.

value assumed for the food requirements of *Calanus* were shown to be ten times too high. The value used was that given by Marshall, Nicholls and Orr (1935) and was calculated from the amount of oxygen taken up by a given number of copepods in unit time. The confirmation of this work was therefore of the greatest importance and we proposed in the present undertaking to repeat the measurements of these investigators, using their own method, and to check them by employing a more precise method. Furthermore, we desired to ascertain whether differences in temperature exert any significant effect on the oxygen requirement of *Calanus* and its application to the nutrition of this species.

Survival and Growth

Material and Methods

For the first group of experiments, which was conducted at Woods Hole during the summer of 1937, copepods were obtained from offshore localities in the vicinity of Cape Cod. In the second group of experiments, which was undertaken at the Harvard Biological Laboratories in Cambridge, material was obtained off Gloucester. In all cases the copepods were collected by towing a scrim net for a few minutes, and were placed in jars which were kept cold during the trip to the laboratory. Immediately upon arrival the animals were transferred by means of a pipette to covered culture dishes and these were set in baths whose temperature was maintained constant to within 0.5° C. In all the experiments except those involving a change of temperatures (see below) the copepods were transferred each day by pipette to freshly prepared culture dishes (each containing 250 cc. of water) which had been brought to the proper temperature.

The sea water used in the Woods Hole experiments was taken freshly from the harbor (except for the experiments of Table II); that used in Cambridge had been collected at Nahant several months previously and allowed to stand in carboys. In both instances the water was Berkefeld filtered (except for a few tests at Woods Hole in which unfiltered harbor water was used) and then thoroughly aerated.

In most of the experiments a known concentration of food was provided by adding a measured amount of a "persistent" culture of either the diatom, *Nitzschia closterium* (Plymouth strain) or the green flagellate, *Platymonas subcordiformis*[2] to each culture dish. The amount of organic matter represented by these forms and also by the diatom, *Rhizosolenia* sp., which was abundant in Woods Hole waters during the

[2] Kindly identified for us by Professor G. W. Prescott of Albion College, Michigan.

summer, may be judged from the following determinations of nitrogen kindly carried out for us by Dr. T. von Brand:

Nitzschia	2.5	γN	per	10^6	cells [3]
Platymonas	12.7	"	"	"	"
Rhizosolenia	96.0	"	"	"	"

To ascertain whether the number of animals present or the concentration of food organisms was sufficient to affect significantly the dissolved oxygen or the pH of the culture medium, tests were carried out on a representative series of culture dishes as indicated in Table I. The

TABLE I

Tests of pH and O_2 in culture dishes with different numbers of copepods and of food organisms.

Concentration of Nitzschia cells/cc.	Number of Stage V Calanus	pH		O_2 cc./liter	
		Initial	After 24 hrs.	Initial	After 24 hrs.
0	0	8.09	7.86	4.25	5.67
0	10	8.09	7.81	4.25	6.42
30,000	0	8.11	9.09*	4.68	5.94
30,000	10	8.11	7.73	4.68	6.65
300,000	0	8.17	8.10	4.81	6.49
300,000	10	8.17	8.00	4.81	6.37

* This value shown by other tests to be atypical.

results show that sufficient O_2 was present in all containers and that the pH was only slightly altered. The slight increase in O_2 content and decrease in pH in the control was due to incomplete temperature equilibrium initially.

Experiments

The first set of experiments was designed to ascertain whether the *change* in temperature which results from the removal of the culture dishes from the constant temperature bath for daily inspection and transfer of animals has a deleterious effect on the copepods. For this purpose groups of *Calanus* and also of *Centropages typicus*—another copepod common in the Woods Hole region—were divided into two parts and placed in culture dishes in constant temperature baths maintained at either 8° C. or 13° C. One culture dish in each group was left undisturbed in the bath as a control but others were removed for varying lengths of time as indicated in Table II. For the duration of

[3] The reason why this value is larger than that found the year before and reported by Fuller (1937) is not known. Variations of this sort must be taken into consideration in calculating the potential food value of each species.

the experiments the survival of *Calanus* was better than that of *Centropages* but in neither case was survival in the control dishes significantly better than that in the dishes which were subjected to a daily temperature change. We conclude, therefore, that contrary to the suggestion of Gross (1937), the daily inspection at room temperature is not harmful in our experiments with these copepods.

Our next set of experiments was designed to test the effect of the concentration of food organisms on the survival and growth of *Calanus* at temperatures of 7° or 8° C. For this purpose culture media were prepared in which the concentration of food organisms differed by

TABLE II

Effect of *change* of temperature on survival. Comparison of number of deaths in cultures removed from constant temperature bath for daily periods of varying lengths with number of deaths in cultures not removed. Two hundred and fifty cc. Berkefeld filtered sea water from tap in each culture dish, left unchanged, and no food added. Experiments carried out in July, 1937 at Woods Hole.

Species	Initial no. of animals in each culture dish	Temp. of constant temperature bath	Period culture removed from bath each day	Temp. of culture when replaced	Duration of experiment	No. of deaths
		°C.		°C.	*days*	
Calanus (Adults)*	10	8.5	30 minutes	14.0	8	1
" "	10	8.3	not removed		8	3
Calanus (Stage V)†	15	8.0	60 minutes	16–19	7	0
" "	15	8.0	not removed		7	1
Centropages (Adults)*	20	8.5	30 minutes	12–15	8	17
" "	22	8.5	120 "	16–21	8	15
" "	20	8.2	not removed		8	12
" "	20	13.3	30 minutes	15–18	8	18
" "	20	13.3	120 "	20–21	8	20
" "	20	13.0	not removed		8	18

* Animals obtained at Whistle Buoy at western entrance to Vineyard Sound.
† Animals obtained at South Channel, east of Nantucket.

very large amounts (Table III). Ten to twenty-five *Calanus* were introduced into each culture dish and their condition noted each day for the duration of the experiments (2 to 4 weeks). The daily transfer of animals to freshly prepared culture media prevented an appreciable change in the concentration of the food from being brought about by either the grazing of the animals or the multiplication of the food organisms. Accumulation of metabolites was similarly avoided.

In this set of experiments no copepods were observed to moult successfully but certain individuals reached the moulting stage and died during the casting of the shell. As in previous investigations, the

number of animals which began the moulting process may be taken as an index of growth. The reliability of this procedure is strengthened by the fact that low numbers of "deaths in moult" are usually accompanied by high numbers of "deaths not in moult" and vice versa (see Table III). On this basis our results reveal the best nutritional conditions in those cases where 30,000 cells/cc. of *Nitzschia,* or more, were provided, but a concentration as high as a million and a half cells/cc. appeared harmful. The moulting stage was attained (i.e. "deaths in

TABLE III

Effect of concentration of food on survival of *Calanus*. Temperature of constant temperature bath for experiments in July and August: 8° C., in September: 7° C. Each group of animals placed in 250 cc. of culture medium. Animals in copepodid Stage V. Experiments conducted at Woods Hole in 1937.*

Food organism	Conc. of food	Exper. begun	Exper. concluded	Initial no. of copepods	Deaths in moult	Deaths not in moult
	cells/cc.					
Nitzschia	300,000	July 11	Aug. 9	15†	8	5
"	150,000	"	"	15	10	4
"	30,000	"	"	15	8	3
"	15,000	"	"	15	4	8
none		"	"	15	4	8
Nitzschia	1,500,000	Aug. 15	Aug. 31	25‡	0	25
"	150,000	"	Sept. 3	25	1	13
"	1,500	"	"	25	4	16
none		"	"	25	0	11
Unfiltered	harbor water	"	"	25	0	22
Platymonas	18,000	Sept. 11	Sept. 31	10‡	0	7
"	4,500	"	"	10	0	6
Nitzschia	230,000	"	"	10	0	9
none		"	"	10	0	6

* Experiment begun September 11 carried out with the assistance of Mr. Dean F. Bumpus, biological technician for the Woods Hole Oceanographic Institution.

† Animals obtained from South Channel, east of Nantucket.

‡ Animals obtained from Cape Cod Bay, 4 miles NNE of Canal.

moult") in a higher proportion of cases in the tests begun on July 11 than in those begun on August 15 and in the experiment of September 11 not a single copepod reached the moulting condition. Indication that the animals stopped feeding toward the end of the season arises from the fact that no significant difference was observed in the survival in different food concentrations for the September 11 experiment. Moreover, in this case and in the experiment of August 15 the unfed controls fared as well or better than the copepods which were provided with

food. This suggests that the feeding or the survival (or both) of the copepods may depend upon the season because of differences in the phase of the life cycle characterising the copepods at the time the experiments were begun (see below).

With the possibility of a seasonal effect in mind, arrangements were made for the next set of experiments to be conducted during the spring months when *Calanus* off our shores is known to be breeding and grow-

TABLE IV

The effect of temperature and food concentration on the survival and growth of *Calanus*. Animals obtained off Gloucester. Each group placed in 250 cc. of culture medium. Experiments conducted at Harvard Biological Laboratories in 1938 with the assistance of Mr. Dean F. Bumpus.

Jar	Temp.	Conc. of Nitzschia	Initial population April 22. Copepodid stage	Successful moults	Deaths in moult	Deaths not in moult	Final population May 18. Copepodid stage
		cells/cc.					
1	3° C.	150,000	10 IV	5	0	1	1 IV, 7 V†
2		1,500	8 IV, 2 III	4	0	2	2 IV, 6 V
3		0	9 IV, 1 III	5	0	3	2 IV, 4 V†
4	6° C.	150,000	11 IV	10	0	5	3 V, 3 VI ♂
5		1,500	10 IV	6	0	5	3 V, 1 VI ♂, 1 VI ♀
6		0	8 IV, 2 III	0	1	9	0
7	9° C.	150,000	9 IV, 1 III	7	1	4	1 V, 1 VI ♂, 2 VI ♀ †
8		1,500	10 IV	5	1	5	3 V, 1 VI ♀
9		0	10 IV	2	1	8	1 V
10*	3° C. then 9° C.	1,500	10 III	4	1	6	2 V†
11	6° C.	1,500	10 III	2	2	7	1 V
12	9° C.	1,500	10 III	6	0	1	?‡

* At the end of 7 days at 3° C. no animals had moulted and none had died. The jar was then transferred to 9° C.

† One animal missing.

‡ Animals accidentally lost on the seventh day.

ing (Fish, 1936; Clarke and Zinn, 1937). In nine tests at three temperatures and with varying amounts of food using copepodid stage IV and in three tests using copepodid stage III, run in exactly the same manner as the experiments of the previous summer, survival was good and a considerable amount of moulting took place (Table IV). In the case of stage IV the number of successful moults at 3° C. did not differ materially with the amount of food provided, but at the other

temperatures a distinct positive relation was found between the growth of the animals and the concentration of *Nitzschia*. The largest number of animals moulting occurred in the culture with high food concentration at 6° C. (Jar 4). Deaths during moult took place chiefly at the high temperatures. Deaths not in moult were much more abundant in the cultures not provided with food and were confined largely to the higher temperatures.

Of the stage III animals, all of which were provided with the same amount of food, the group at 9° C. was accidentally lost on the seventh day but six in this group had already moulted. In contrast, there were no signs of growth by the seventh day in the group at 3° C. However, after the latter animals had been transferred to the 9° bath, four moults occurred within six days. In the culture at 6° C. there were two successful moults and two deaths in moult. Three of the stage III animals attained stage V, thus having passed through two moults. In the case of the copepods which were in stage IV at the beginning of the experiment, nine individuals (in the 6° and 9° cultures) moulted twice and thus reached the adult condition (stage VI).[4] From the results of this set of experiments we may conclude that at 3° C. moulting was retarded but at the same time survival was good. A larger number of successful moults and deaths in moult occurred at 6° C. and about the same proportion at 9° C. but at these temperatures survival was definitely inferior to that at 3° C.

Respiration

Material and Methods

The copepods used for the experiments on respiration were taken by tow net in Cape Cod Bay and placed in large jars packed in ice for the return run to the laboratory. Vigorous specimens of copepodid stage V were then picked out by pipette, transferred to culture dishes and placed in the refrigerator at 8° C. until the tests were begun on the following day. The sea water used for the cultures and for the experiments was taken in carboys at the point where the animals were collected (except for the tests at 16.8° C. in which the laboratory salt water supply was used). In all cases the sea water was Berkefeld filtered and aerated before use. No food was added.

Respiration was measured by the Winkler method and in other tests by a manometric method in which a respirometer of the Dixon-Haldane constant pressure type was used. In the former case almost exactly the same procedure was followed as that described by Marshall, Nicholls, and Orr (1935) except that in the tests reported below 25 and

[4] Under the microscope the gonads of both sexes were observed to be ripening at the end of the experiment.

75 animals were used respectively in 200 cc. of water instead of the 120 animals employed by the earlier workers. In the latter case the apparatus was arranged as outlined by Dixon (1934) with the exception that no KOH well for the absorption of CO_2 was used. Tests showed that no difference in the changes of value of the gas within the apparatus occurred when the KOH well was removed. It is probable that over the short periods of time involved the small amount of CO_2 produced was readily absorbed by the slightly alkaline sea water. In our apparatus all joints were of ground glass and were carefully sealed with stopcock

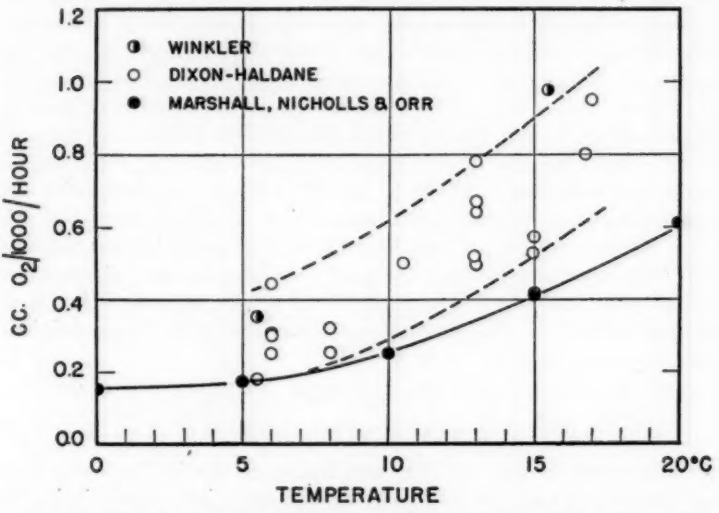

FIG. 1. Relation between temperature and amount of oxygen consumed by stage V *Calanus*. Dashed lines give the limits of all determinations with the Dixon-Haldane respirometer. Experiments carried out at Woods Hole during August and September, 1938.

grease or vaseline. The respiration flasks were completely immersed in a constant temperature bath controlled to 0.5° C.

In the experiments which follow 6 to 30 animals were placed in 10 to 20 cc. of water in one of the respiration flasks. This flask had been painted black to exclude light since Marshall, Nicholls, and Orr reported that differences in illumination exerted a marked influence on the rate of respiration. Equilibrium between the liquid and the gas above it was attained by attaching the entire apparatus to a Warburg shaker. Since continuous or rapid shaking resulted in poor survival of the copepods and might influence abnormally the rate of respiration, the

shaking was limited to the 5 or 10 minutes immediately before each reading and was confined to an amplitude of 2 cm. at a frequency of 100/min. Readings were ordinarily made every 20 minutes for 4 or more hours. The change in volume for each period was observed and reduced to cc. dry gas at N. T. P. Blank trials, which were run at intervals in exactly the same way but with no animals, showed no variation in volume over periods as long as 4 hours. In certain cases water in which copepods had been living and which presumably contained

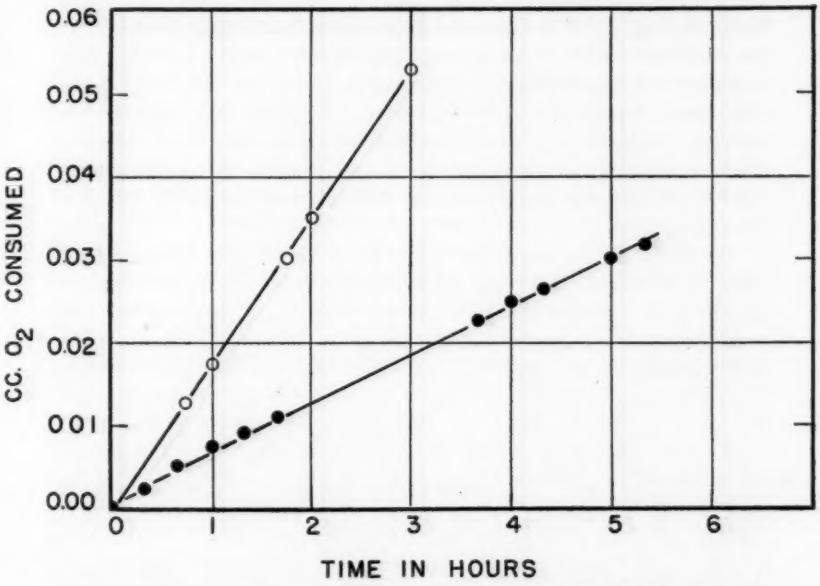

FIG. 2. Progress of two typical experiments on consumption of oxygen by stage V *Calanus*. Closed circles: 6 animals at 16.8° C. Open circles: 28 animals at 13.0° C.

a characteristic number of bacteria was used for the blank tests. Thus assurance was provided that in the experiments neither microörganisms nor other extraneous agents were interfering appreciably with the results.

Experiments

Two measurements of the oxygen consumption of *Calanus* were made using the Winkler method. The first of these was carried out at 5.5° C. using 25 animals confined in 200 cc. of sea water for 4 hours and yielded a value of 0.35 cc. O_2/hr./1,000 copepods. In the second

case 75 animals in 200 cc. were tested at 15.5° C. for 4 hours and were found to have consumed oxygen at the rate of 0.98 cc. O_2/hr./1,000 copepods. The first of the values is somewhat higher and the second is considerably higher than those obtained by Marshall, Nicholls, and Orr (1935), as may be seen in Fig. 1.

The experiments using the Dixon-Haldane respirometer comprised 17 tests carried out at 7 temperatures ranging from 2.5° C. to 16.8° C. In each test from 4 to 12 readings were made during the course of the run and these were plotted against time, as in the two typical experiments shown in Fig. 2. The fact that these curves and all the others which have been used in the calculations exhibited only a slight deviation from a straight line shows that the consumption of oxygen was sensibly constant over the period of each experiment. Marshall, Nicholls, and Orr reported a drop in oxygen consumption during the first 10 or 15 hours after the capture of the copepods but in our experiments, since measurements were not begun until the day following capture, this period of decreasing respiration was avoided.

From the average slope of each curve of oxygen consumption a value was obtained which was converted into cc. O_2/hr./1,000 copepods and the results plotted in Fig. 1. It will be observed that a considerable variation exists among the several tests at the same temperatures. Evidently respiration in *Calanus* can vary between wide limits and this conclusion is borne out by the observations of the earlier investigators. However, there is no doubt as to the general magnitude of the oxygen requirement of *Calanus* in the present experiments. The influence of temperature on respiration is indicated by the upward slope of the dashed lines in Fig. 1 enclosing all the points. The values increased from an average of about 0.32 cc. O_2/hr./1,000 copepods at 5.5° C. to about 0.91 cc. O_2/hr./1,000 copepods at 16.8° C. Although our measurements give a somewhat higher average rate of respiration at all temperatures than was obtained by Marshall, Nicholls, and Orr, they are of the same order of magnitude. Furthermore, there is no indication of a possible lower oxygen requirement for the Woods Hole copepods since *every one* of the present values was higher than those obtained by the British investigators.

Discussion

The foregoing experiments not only have confirmed and illuminated the early work at Woods Hole, but in addition have raised a number of new questions. It is clear that both the amount of food and the temperature have an important influence on the growth of *Calanus* and on

its survival.[5] The situation is further complicated by the fact that under conditions which tend to promote growth, survival may be poor because of the likelihood of death during the moulting process. The provision of food improves growth and survival, but the exact amount of food seems not as important as originally supposed (cf. Clarke, 1939). Although a minimum was ordinarily required for growth, in certain experiments at low temperatures some moulting was observed in the entire absence of food. And in at least one case a very high concentration of food was found to be harmful (see also Lucas, 1936).

It is a striking fact that a large amount of moulting took place and good survival was observed in our experiments conducted in April and May (Table IV) whereas poor survival and no successful moulting was the rule for the tests made during the summer and early autumn (Table III) and for the summer experiments of Fuller (1937, Table V, temperature 13° C.).[6] In all cases *Nitzschia* was used as the food organism in at least some of the cultures, the temperatures of the cultures were not widely different, and exactly the same procedure was followed. For the April and May tests copepods were obtained from a point off Gloucester but in two of the other cases the experimental animals were procured from a location which was also north of Cape Cod although considerably to the south of Gloucester.[7] If the place of origin of the copepods is immaterial, it might be suggested that the time of year at which the animals were collected could have an important bearing on the laboratory results. According to the observations of Clarke and Zinn (1937) and of Fish (1936) the second of the two yearly broods of *Calanus* grows to copepodid stage V during the summer and remains in that stage until the following January or February. It seems possible that in nature feeding may be reduced to a minimum and mortality may be especially high during the late summer and that animals collected during this period might exhibit poor survival and refuse to grow even under the most favorable laboratory conditions. Added support for this view is derived from the fact that in our experiment begun September 11 (Table III) no successful moults occurred and no animals were observed even to attempt to moult.

[5] In fact Fish (1937) is of the opinion that the presence of suitable temperatures is the most important factor in the production of zoöplankton in the Gulf of Maine and the Bay of Fundy.

[6] In the earlier summer experiments of Clarke and Gellis (1935) and of Fuller and Clarke (1936) growth and survival were better, but the different methods used make comparisons inappropriate.

[7] The following ocean temperatures were observed:

Off Gloucester, April 26, 1938, 9.6° C. at surface.

Cape Cod Bay, August 14, 1937, 18.3° C. at surface, 10.2° C. at 15 m., 8.6° C. at 30 m.

South of Cape Cod temperatures were generally higher.

In all of our experiments at Woods Hole and especially in those described above the copepods were noticed to be heavily parasitized immediately after death. An animal which appeared perfectly healthy one day would be found dead on the next day with its body filled with opaque whitish or colored masses. Possibly parasites gain a foothold within the copepod and remain unnoticed until they suddenly reach some vital tissue. From this initial infection the rapid and obvious spread of the parasite to all parts of the body could be accomplished after death within a relatively short interval. Since Jepps (1937) reported that a large proportion of *Calanus* population was found to be infected when collected, it is possible that the inroads of parasites may be an important element underlying the fluctuation in the abundance of this species both seasonally and annually.

The experiments on respiration show that the values which have been used for the oxygen consumption of *Calanus* and hence its food requirement, are of the correct order of magnitude. In fact, our measurements indicate that an even larger amount of food per day is required by these organisms than was assumed by Marshall, Nicholls, and Orr. Therefore, the discrepancy between the nutrient required and the number of diatoms available for food gapes wider than ever. According to Harvey (1937) *Calanus* can filter out the larger species of diatoms much more effectively than the smaller forms. He found that 100 times the volume of water may be swept free in an hour when *Lauderia* or *Ditylium* were provided as food as when *Nitzschia* was used. However, in nature the larger diatoms are not as abundant as the more minute species, and a careful investigation of the amount of food per cc. of sea water which the scarcer but more bulky types would provide is seriously needed. Another point which needs study is the " micro-distribution " of the phytoplankton and nannoplankton. Although uniformity in abundance may be found in a series of one-liter water samples taken from an area, the distribution *within* each liter may be highly irregular. If the food organisms occur in clusters, and if a copepod can find and stay in a local though minute zone of abundance, sufficient nutriment could be obtained without the necessity of filtering an excessive volume of water. Such explanations as the foregoing may resolve the discrepancy between the daily food requirement and the *average* abundance of potential food organisms.

Summary

Laboratory tests on cultures of *Calanus* in relation to the ecology of this species showed that: (a) removal of the culture dishes from constant low temperature to room temperature for daily periods as great

as 120 minutes was not harmful to the copepods; (b) growth was poorer at 3° C. than at 6 to 9° C. but survival is better at the lower temperatures; (c) both growth and survival decreased regularly in passing from experiments conducted in the spring to those in the autumn.

Measurements of the respiration of *Calanus* using the Winkler method and the Dixon-Haldane respirometer showed that the magnitude of the oxygen requirement for our animals is of the same order as for those tested by Marshall, Nicholls, and Orr, and possibly is higher than previously reported. The discrepancy between the estimated food requirement of *Calanus* and the average abundance of diatoms therefore still exists but certain possible explanations are discussed.

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DEVELOPMENT OF HALF-EGGS OF CHAETOPTERUS PERGAMENTACEUS WITH SPECIAL REFERENCE TO PARTHENOGENETIC MEROGONY

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It has been shown in previous studies on sea urchin eggs (1936, 1938) that the non-nucleate fractions, obtained by centrifugal force, may be activated with parthenogenetic agents and may cleave and develop, without nuclei, to the blastula stage. This I have termed parthenogenetic merogony. Five species of sea urchins have given similar results. I had hoped that the parthenogenetic merogone of another class of animal might develop further than the blastula, and for this reason have made the present study.

The egg of the worm, *Chaetopterus,* fulfills the two requirements necessary for parthenogenetic merogony. First, it can be broken apart readily by centrifugal force so that one can obtain non-nucleate fractions in abundance, and second, the normal egg can easily be made to develop parthenogenetically by treatment with salts. Although my chief concern has been the development of the parthenogenetic merogones, a study has also been made of the fertilized merogones for comparison, as well as of the whole centrifuged egg and the white (nucleate) halves both fertilized and parthenogenetic. Although some of my observations may seem to repeat the earlier ones of Lillie (1906, 1909) and Wilson (1929, 1930), the present study is on fractions of a definite size and known nuclear and cytoplasmic composition whereas previous observations have been made on fragments of irregular size and composition and of assumed nuclear content. Certain differences in my results as compared with previous work may be attributed to this difference in material. Other discrepancies are due to the greater centrifugal force used in these experiments, but particularly to the fact that the eggs have been centrifuged in a medium of cane sugar and sea water. The sugar changes the surface of the egg, and in fact sometimes acts on these eggs as a parthenogenetic agent, similar to KCl. A sugar solution was used as a medium because it can be made, by the addition of the proper amount of sea water, of the same specific gravity as the eggs (as well as, by proper dilution, of the same osmotic pressure), so that the eggs remain suspended during centrifugation, and are free to break into two

halves. It also permits the eggs to orient with the lighter area, containing the polar spindle, toward the centripetal pole of the centrifuge, whereas in sea water alone the heavy eggs are quickly thrown to the bottom of the centrifuge tube and are held in the position in which they are thrown. Although no special study has been made of the relation of polarity of the egg and the cleavage planes to the stratification and plane of breaking caused by the centrifugal force, there seems no doubt from observations with the centrifuge microscope that the eggs in the sugar solution *do* orient with the polar area toward the centripetal axis of the centrifuge. It may be mentioned in passing that the *Arbacia* egg in the 12-cell stage, when the colorless micromeres appear, also orients with the micromeres toward the centripetal pole of the centrifuge; this can be readily observed with the centrifuge microscope. The lack of pigment granules makes these cells lighter than the other cells, which are pigmented. In the *Chaetopterus* egg, the clear polar region of the egg when ready for fertilization (Photograph 18) is lighter than the granular region and therefore the egg orients as stated above. When the eggs are centrifuged immediately on laying, in the germinal vesicle stage (Photographs, 17, 3), there is no visible lighter area, and there is no apparent orientation. This is, I think, in line with Morgan's studies (1937, 1938); he has definitely determined that the germinal vesicle is not eccentric even in eggs still in the parapodia; *it* therefore cannot cause orientation.

Stratification and Breaking

(Photographs 1–16)

The *Chaetopterus* egg stratifies readily. In fact, simply leaving the eggs undisturbed over night in isotonic KCl produces a very nice stratification (Photograph 1). This is true also for the *Arbacia* egg to a slight extent. The *Chaetopterus* egg, both unfertilized and fertilized, stratifies also readily with centrifugal force (Photograph 2). It stratifies similarly to the *Arbacia* egg except that there is no pigment; the stratified egg is almost exactly like that of another sea urchin, *Sphaerechinus granularis*. Some photographs of the stratified eggs of the annelid, *Chaetopterus*, and of the echinid, *Spaerechinus*, are very similar and might easily be mistaken the one for the other except that there is a little more oil in the *Chaetopterus* egg (Cf. Photographs 4 and 5). The unfertilized eggs of *Chaetopterus* were usually in these experiments centrifuged after the polar spindle had formed; it was found difficult to stratify them well (Photograph 3) and almost impossible, with the forces used, to break them apart in the very short interval elapsing

between laying and the breaking of the germinal vesicle. The oil goes to the light pole, then there is a clear layer, then a band of mitochondria which stains purple with methyl green, and then the yellow yolk granules at the heavy pole. (Photographs 4, 6). The nucleus, or rather the first polar spindle, *always* lies in the clear zone under the oil. The eggs are very heavy and are usually thrown to the bottom of the centrifuge tube in the same sugar solution in which *Arbacia* eggs are nicely suspended. The result is that the egg breaks irregularly; usually a small oil cap is thrown off and the rest of the egg breaks into irregular fragments (Photographs 13, 14 of eggs in the centrifuge microscope slide). This is also the case when the eggs are centrifuged in sea water alone. The earlier experiments of Lillie and Wilson and most of Morgan's experiments were done in this way.

It is possible, however, to keep the eggs suspended during centrifugation by using a minimum of sea water with the eggs, and a large amount of the isotonic sugar solution. In this way, after centrifuging

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PLATE I

Stratification and Breaking of Eggs

The photographs were all taken of living eggs with a Leica camera, and for the most part with a water immersion (× 40) lens. The magnification on the plates with the exception of Plate II is approximately 175 ×; Plate II is approximately 250 ×. The smaller photographs were taken with a low power (× 10) lens and are here magnified about 65 ×. The times (i.e., minutes, hours) given are derived from the data recorded at the time of photographing.

PHOTOGRAPH 1. *Chaetopterus* egg kept overnight undisturbed in isotonic KCl.

PHOTOGRAPH 2. Egg centrifuged (after fertilization) for comparison with Photograph 1.

PHOTOGRAPH 3. Egg centrifuged immediately on laying; shows unbroken germinal vesicle; this broke immediately afterwards.

PHOTOGRAPH 4. Stratified *Chaetopterus* egg; oil, clear layer, mitochondria and yolk. Polar spindle is in the clear layer under the oil.

PHOTOGRAPH 5. Stratified egg of *Sphaerechinus granularis* for comparison with Photograph 4; same layers.

PHOTOGRAPHS 6–9. Breaking apart of stratified egg of *Chaetopterus* into two halves of uniform size.

PHOTOGRAPH 10. White half-eggs, containing oil, clear layer, mitochondria and a little yolk; also polar spindle. Note uniformity of size.

PHOTOGRAPH 11. White half-egg showing band of mitochondria; stained with methyl green.

PHOTOGRAPH 12. Yellow half-egg, containing yolk.

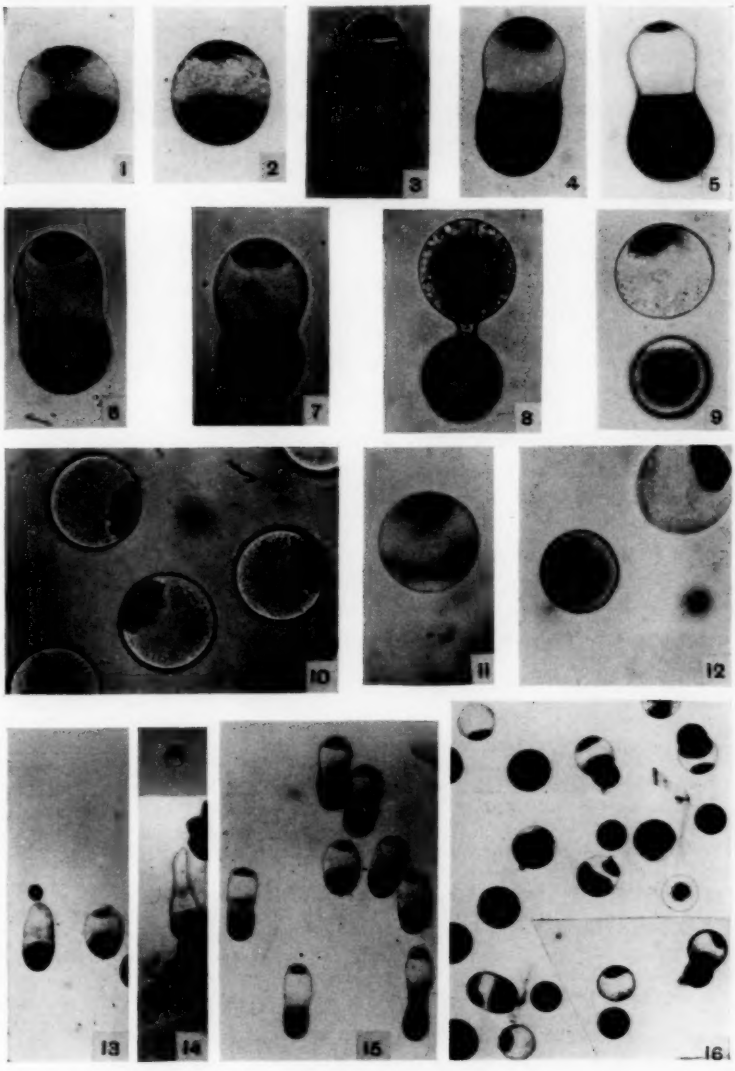
PHOTOGRAPHS 13–15. Eggs immediately after removal from the centrifuge microscope, and still in the centrifuge microscope slide.

PHOTOGRAPHS 13, 14. Eggs supported at bottom of slide. Oil cap pulls off and egg breaks irregularly.

PHOTOGRAPH 15. Eggs suspended in sugar solution. Ready to break into halves of uniform size.

PHOTOGRAPH 16. A group of eggs just removed from the centrifuge tubes, showing regularity of breaking and uniformity of size of halves. The larger black spheres are the whole eggs viewed from above.

PLATE I



Photographs 1–16

for five minutes at 10,000 × g., the eggs stratify, become elongate, then dumbbell shape, and then break into spheres, quite regular in size and quite similar to the halves of the *Arbacia* egg (Photographs 6–9, 16; 15 in the centrifuge microscope slide). The *Chaetopterus* egg breaks, so that the lighter or white half contains oil, clear layer, nucleus (or polar spindle), mitochondria and a little yolk (Photographs 9, 10, 11). The heavier yellow half contains only yolk (Photographs 9, 12). The measurements for the whole and half eggs are:

Whole egg	Diameter	94 μ	Volume	434900 μ^3	
White half	"	83 μ	"	299400 μ^3	436700 μ^3
Yellow half	"	64 μ	"	137300 μ^3	

The white half has over twice the volume of the yellow half. Many batches of eggs have given similar figures.

Normal Development

(Photographs 17–31)

As is well known, especially from the early work of Wilson (1883) and Mead (1895, 1897, 1898 *a*, *b*), the early development of the egg is as follows. The egg is laid in the germinal vesicle stage (Photograph 17); after resting in sea water a few minutes, the germinal vesicle

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PLATE II

Normal Development of the Chaetopterus Egg

PHOTOGRAPH 17. Immature egg immediately on laying; germinal vesicle still intact in center.

PHOTOGRAPH 18. Egg 15 minutes after laying; polar spindle in white area at upper pole.

PHOTOGRAPH 19. Fertilized egg, showing crinkled or fluted fertilization membrane. Twenty-five minutes after fertilization.

PHOTOGRAPH 20. Egg flattened where first polar body is being extruded. Fifteen minutes after fertilization.

PHOTOGRAPH 21. Egg rounded again after first polar body has been extruded; note fluted membrane near polar body. Twenty-five minutes after fertilization.

PHOTOGRAPH 22. Egg flattened again at time of formation of second polar body. Thirty minutes after fertilization.

PHOTOGRAPH 23. Same egg 1 minute later; it has again rounded out.

PHOTOGRAPH 24. Pear-shaped stage. Thirty-four minutes after fertilization.

PHOTOGRAPH 25. Same egg 2 minutes later; beginning of polar lobe.

PHOTOGRAPH 26. Same egg 4 minutes later; 40 minutes after fertilization. First cleavage beginning, with polar lobe still present.

PHOTOGRAPH 27. Same egg 20 minutes later; first cleavage complete and polar lobe withdrawn; note polar bodies near furrow at upper pole.

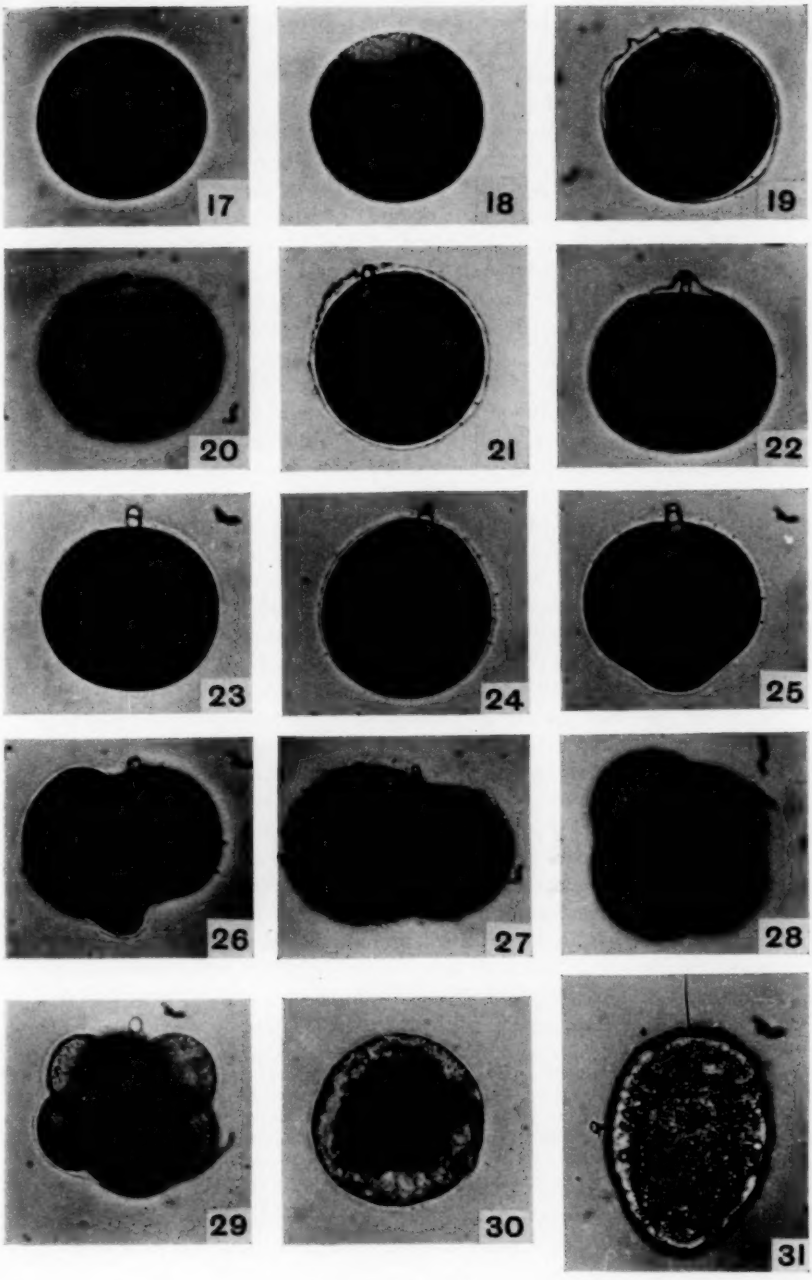
PHOTOGRAPH 28. Four-cell stage, 1 hour, 40 minutes after fertilization. Note characteristic *Brechungslinie* or cross-furrow.

PHOTOGRAPH 29. Eight-cell stage, 2 hours after fertilization.

PHOTOGRAPH 30. Blastula, just before swimming; 4½ hours after fertilization.

PHOTOGRAPH 31. Trochophore, 1 day after fertilization.

PLATE II



Photographs 17–31

breaks and the first polar spindle forms. The polar region is seen in the living egg as a clear area near the surface; it is fully formed (at 23° C.) 10–20 minutes after laying (Photograph 18). The egg remains in this condition, with the first polar spindle in the metaphase, indefinitely unless fertilized. If fertilized at this stage, in about 15 minutes [1] the first polar body appears (Photograph 20). The fertilization membrane is the same, according to Whitaker (1933), as the vitelline membrane which lifts from the surface. It is peculiar and characteristic for this egg and quite different from the fertilization membrane of sea urchin eggs. It is crinkled or fluted especially in the region of the polar bodies (Photographs 19, 21). The egg becomes markedly flattened at the pole at the formation of the first polar body (Photograph 20); then becomes spherical (Photograph 21). Then the second polar body is formed, again with a slight flattening (Photograph 22)—about 30 minutes after fertilization; and the egg once again rounds out (Photograph 23). Soon after this (35 minutes after fertilization) the egg becomes pointed or pear-shaped with the narrow end toward the polar bodies (Photograph 24). After again rounding out, the characteristic polar lobe is formed (about 5 minutes after the pear-shape) at the opposite end (Photograph 25). This remains till after first cleavage which begins about 40 minutes after fertilization (Photograph 26). The first cleavage is very unequal giving a small cell and a large one into which the yolk lobe is absorbed (Photograph 27). At the second cleavage, 1½ hours after fertilization, the small cell divides equally and the large cell unequally so that there are two small cells, one large cell and one intermediate in size (Photograph 28), a form of cleavage quite typical for many annelid and mollusk eggs. The next cleavage is shown in Photograph 29, taken 2 hours after fertilization. The spiral type of cleavage which occurs in *Chaetopterus* is difficult to follow in the living egg, so that no attempt has been made to photograph the later cleavage stages of the normal egg, nor to follow them in the experimental work. A normal blastula is shown in Photograph 30, taken just before swimming, which occurs about 5 hours after fertilization; and a one-day trochophore, in Photograph 31.

[1] The times for the various stages are quite variable in the *Chaetopterus* egg even with constant temperature. It differs in this respect from the *Arbacia* egg in which, in the height of the season, the stages occur with almost clock-like regularity at any given temperature. Late in the season (September, October) there is considerable delay in the *Arbacia* egg irrespective of temperature. Eggs may be obtained through November from *Arbacias* which have been brought in before the first part of August and kept in the aquaria in running water. Animals brought in from the sea after August 15 have practically all shed their eggs.

Normal Egg, Parthenogenetic

The parthenogenetic development of the normal uncentrifuged egg by the addition of KCl to the sea water has been described by Mead (1898 *b*), Loeb (1901) and Lillie (1902, 1906), and in detail by Allyn (1912). The peculiar feature is the amoeboid form of the egg and the frequent occurrence of very large nuclei without cell division. The later embryos tend to stick together and form complex multiple organisms as described particularly by Loeb (1901). This is probably due to the effect of the KCl solution on the surface of the cells. Unequal two- and four-cell stages of the characteristic annelid type also occur, and the parthenogenetic egg may give rise to apparently normal swimmers just like the fertilized egg. Some equal two-cell stages also occur.

My best results for parthenogenesis have been obtained by putting the eggs into a solution of 1 gram KCl + 100 cc. of sea water for 30 minutes, then transferring them to sea water. However, I have found other proportions and times of exposure to work better with some batches of eggs. The different batches respond quite differently to the KCl solutions and I could get no dependable standard solution.

Whole Egg Centrifuged, Then Fertilized

(Photographs 32–42)

The *centrifuged* whole egg, which has been elongated by the centrifugal force, becomes spherical again quite quickly after the force is removed, and this whether the egg is fertilized or not. It is thus strikingly different from the sea urchin egg which becomes spherical if left unfertilized, but retains its elongate or dumb-bell shape if fertilized or artificially activated, all through the cleavages up to the swimming blastula. After fertilization of the centrifuged egg, in *Chaetopterus,* the polar bodies usually come off near, but not on top of, the oil cap (Photograph 32); if the oil cap has been centrifuged off, however, they usually come off exactly at the centripetal pole (Photograph 33); the oil cap apparently interferes with the protrusion of the polar bodies. This is shown also by the results of centrifuging the egg immediately on laying, while the germinal vesicle is still intact, as shown in Photograph 3. When, after removal from the centrifuge, the germinal vesicle breaks, it dissipates the oil in clumps (Photograph 34). The polar spindle forces itself to the center of the oil, which now forms a circle of clumps around it (Photograph 35). After fertilization, the polar bodies come off in the center of the ring of oil clumps.

The fertilized centrifuged egg usually passes through the changes in shape characteristic of the normal fertilized egg as described above, in-

cluding the formation of the polar lobe. It often divides unequally and typically in the first cleavage (Photograph 36), and the second cleavage as well as later ones (Photograph 39) may be of the characteristic annelid type as has been described by Lillie (1906) and Wilson (1929). Some of the cleavages, however, especially in strongly centrifuged eggs, are irregular and atypical. The first division plane is usually perpendicular to the stratification (Photograph 36). It may, however, pass parallel with the stratification, separating the egg into a clear and a granular zone (Photograph 37). These zones may develop separately just as in the centrifuged *Arbacia* egg (Harvey, 1932); the upper clear area usually divides into cells but the lower yolk half is characterized by nuclear without cytoplasmic divisions (Photograph 38). Many swimming, apparently normal, larvae have arisen from the fertilized centrifuged eggs (Photograph 40). But the blastulae tend to gather together in groups and fuse with each other and with the white halves,

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PLATE III

PHOTOGRAPHS 32–42. *Eggs centrifuged, then fertilized.* Photographs 43–46. *Eggs centrifuged then treated with KCl; parthenogenetic.*

PHOTOGRAPH 32. Polar bodies at side of oil cap; 23 minutes after fertilization.

PHOTOGRAPH 33. Polar bodies at centripetal pole where oil cap has been centrifuged off. Twenty-five minutes after fertilization.

PHOTOGRAPH 34. Egg was centrifuged immediately on laying (see Photograph 3); germinal vesicle breaks after removal from centrifuge and scatters oil in clumps.

PHOTOGRAPH 35. On formation of polar spindle a few minutes later, the clumps of oil are forced into a ring.

PHOTOGRAPH 36. Normal cleavage of centrifuged egg; cleavage plane at right angles to stratification. Note polar lobe. Fifty minutes after fertilization.

PHOTOGRAPH 37. Cleavage plane parallel with stratification. One and one-quarter hours after fertilization.

PHOTOGRAPH 38. Later stage of Photograph 37; upper cells have cleaved several times, lower cell has not cleaved, but the nuclei have divided. One and three-quarter hours after fertilization.

PHOTOGRAPH 39. Normal development of centrifuged egg. Two and one-half hours after fertilization.

PHOTOGRAPH 40. Same, four and three-quarter hours after fertilization; just before swimming.

PHOTOGRAPH 41. Fusion of whole centrifuged and white half-blastulae. Seven hours after fertilization.

PHOTOGRAPH 42. A pair of these, high power.

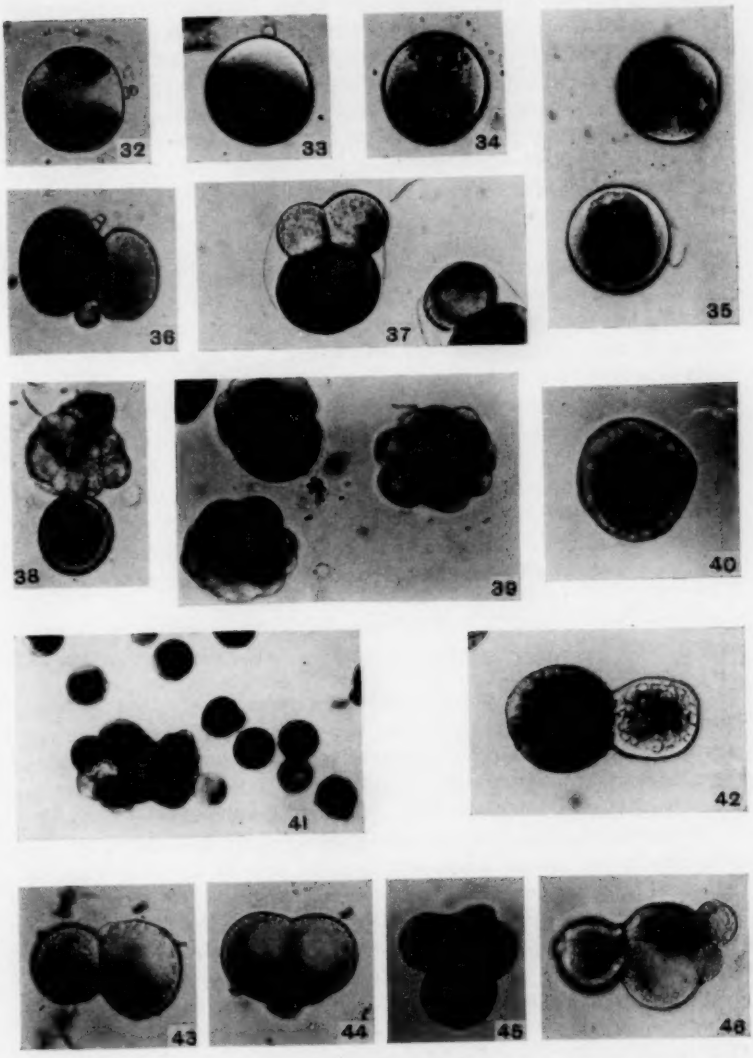
PHOTOGRAPH 43. Two-cell stage of parthenogenetic centrifuged egg; typical unequal blastomeres; cleavage plane perpendicular to stratification. Two hours after treatment.

PHOTOGRAPH 44. Same, equal cells. Note polar lobe.

PHOTOGRAPH 45. Four-cell stage, parthenogenetic. Three hours after treatment. Compare with Photograph 74, of the yellow half-egg fertilized.

PHOTOGRAPH 46. Amoeboid form of parthenogenetic centrifuged egg, 5 hours after treatment. Compare with parthenogenetic white half, Photograph 58; with fertilized yellow half, Photograph 75; and with parthenogenetic merogone, Photograph 96.

PLATE III



Photographs 32–46

forming complex organisms (Photographs 41, 42) just as is characteristic of the parthenogenetic normal eggs. This agglutination must be due to the effect of the sugar solution (in which the eggs have been centrifuged) on the surface of the eggs. It cannot be due entirely to the lack of a fertilization membrane, since eggs from which fertilization membranes have been removed by shaking, and which were kept in sea water, without sugar, do *not* agglutinate.

Centrifuged Egg, Parthenogenetic

(Photographs 43–46)

The centrifuged egg will develop parthenogenetically by the addition of KCl to the sea water, in exactly the same way as the uncentrifuged. Polar bodies may be given off often while still in the KCl solution, and there occur some typical normal cleavages (Photograph 43). But there is a tendency toward equal first cleavage (Photograph 44) and most of the eggs soon become amoeboid and develop with large nuclei and few cell divisions like the uncentrifuged parthenogenetic eggs (Photographs 45, 46). The blastulae tend to fuse together just as do the parthenogenetic normal eggs and the fertilized centrifuged eggs. Development is slower in the parthenogenetic eggs, and swimmers occur in 8 hours instead of 5. This has been found true also of parthenogenetic normal eggs of *Chaetopterus* as well as of many other eggs.

White Half-egg, Fertilized

(Photographs 47–55)

The white half-egg, which contains the polar spindle, can be fertilized, and may develop exactly like the normal whole egg. The egg passes through the flattened and pear-shape phases and the polar bodies are usually given off near the oil cap; these are usually larger than in the normal egg (Photograph 47). The egg usually divides unequally in the first cleavage (Photograph 48, lower egg) and the large cell unequally in the second cleavage giving the typical four-cell stage of two small cells, one large and one intermediate in size (Photograph 49, lower egg). The nuclei are especially striking owing to the lack of granules; these obscure the nuclei in the living whole eggs. There is a tendency for the egg to become amoeboid even before the first division, so that it is not possible to determine if there is a polar lobe or not (Photograph 50). There are often protuberances from one of the blastomeres resembling polar lobes and these occur also in the four-cell stage (Photograph 51). There is also a tendency toward equal first cleavage. Normal blastulae and swimming trochophores are often

formed exactly like those of the whole egg, except that they are white instead of yellow and are smaller (Photograph 52, inset, upper left). But frequently, the blastulae have irregular sized cells and large nuclei like the parthenogenetic whole eggs and they tend to become amoeboid (Photograph 52). The blastula shown in Photograph 53 was both amoeboid and ciliate. These white blastulae often stick together in clumps and fuse with each other and with the whole blastulae present in the same lot (Photographs 54, 55, 41, 42). As pointed out above, this is probably due to the effect of the sugar solution on the surface. The development of the fertilized white halves takes place at about the same rate as the whole eggs; swimmers occur in 5 hours.

The striking thing is that the white half-eggs often cleave, at least in the early stages, exactly like the whole eggs in the very peculiar and characteristic pattern typical of the annelid egg. This has been found also to be the case in many irregular fragments studied by Lillie (1909) and Wilson (1929).

White Half-egg, Parthenogenetic

(Photographs 56–62)

The white half-eggs may be activated by KCl similarly to the whole eggs; some have been activated and have produced swimmers without further treatment than the sugar solution in which they were centrifuged. They may throw off polar bodies often while still in the KCl solution (Photograph 56) and may cleave in the typical annelid fashion (Photograph 57), at least to the four-cell stage. But they, like the whole parthenogenetic eggs, often divide equally at first cleavage, and they usually become amoeboid and develop with irregular cells and large nuclei (Photographs 58–62); these irregularities are much more pronounced in the parthenogenetic than in the fertilized white halves. Development is also delayed as is usual with parthenogenetic eggs.

Yellow Half-egg, Fertilized (*Fertilized Merogone*)

(Photographs 63–84)

The yellow half-egg, which contains no nucleus, may be fertilized and the fertilization membrane is lifted off. I have observed no polar bodies of which I could be sure, though there are often clear amoeboid protrusions simulating polar bodies. One would not, of course, expect polar bodies in this half-egg, since the polar spindle is always in the other half. There is also no polar lobe of which one can be sure. There is usually no cleavage of this half-egg, but it becomes amoeboid and may live in this condition for several days. In some eggs, I have

found, several hours after fertilization, a nucleus, from the sperm, and later two nuclei, and much later several nuclei of different sizes—that is, nuclear division without cell division (Photographs 63–65), such as is characteristic of the heavy red halves of the *Arbacia* egg (Harvey, 1932). However, several eggs have given a typical first cleavage of two unequal cells (Photographs 66, 67). Several eggs have divided into two equal cells, and in one case these divided again equally giving four equal cells—not typical for this form (Photographs 68, 69). These four cells, later on, fused, cleaved again, became amoeboid and soon went to pieces (Photographs 70–75). In general a failure to cleave and a tendency to become amoeboid are characteristic of the fertilized yellow halves or fertilized merogones. No blastulae or ciliated structures from the fertilized yellow halves have been observed. A series of photographs (76–84) will show the extreme amoeboid activity of one of these eggs in the two-cell stage. These photographs

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PLATE IV

White half-eggs, fertilized (47–55) *and parthenogenetic* (56–62)

PHOTOGRAPH 47. White half fertilized, with polar bodies. Twenty-five minutes after fertilization.

PHOTOGRAPH 48 (lower egg). Typical two-cell stage. One and one-quarter hours after fertilization.

PHOTOGRAPH 49 (lower egg). Typical four-cell stage. One and one-half hours after fertilization. Note *Brechungslinie* or cross-furrow as in Photograph 28. In upper egg, all the oil is in one blastomere, and the blastomeres are not quite typical in size.

PHOTOGRAPH 50. Just before first cleavage, amoeboid at position for polar lobe. Fifty minutes after fertilization.

PHOTOGRAPH 51. Four-cell, amoeboid. One hour after fertilization.

PHOTOGRAPH 52. Amoeboid blastulae with large irregular nuclei, and also normal white blastula (inset, upper left). At the upper right is a normal whole blastula (centrifuged). Five hours after fertilization.

PHOTOGRAPH 53. White blastula, amoeboid and also ciliated. Six and one-half hours after fertilization.

PHOTOGRAPH 54. White blastulae fused together. See also Photograph 41. Six hours after fertilization.

PHOTOGRAPH 55. A pair of fused blastulae, high power. See also Photograph 42.

PHOTOGRAPH 56. Parthenogenetic white half with two polar bodies. One hour after activation.

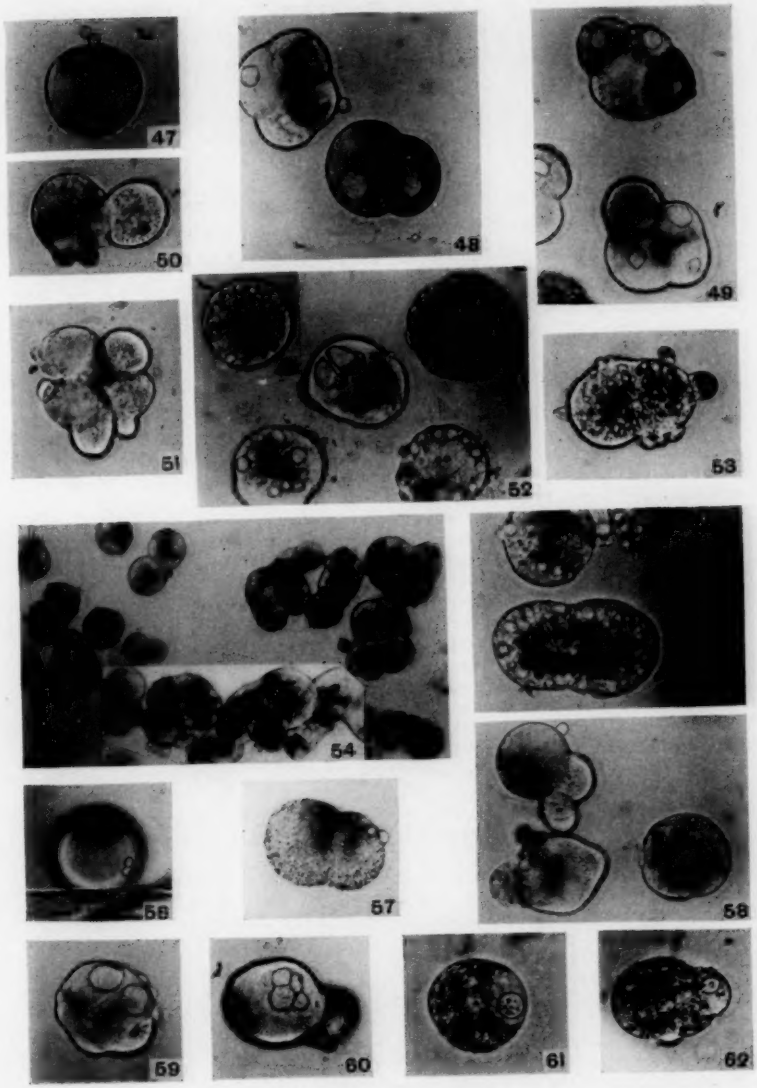
PHOTOGRAPH 57. Typical two-cell stage of parthenogenetic white half. Two hours after activation.

PHOTOGRAPH 58. Parthenogenetic white halves, amoeboid. One and one-half hours after activation. Cf. with Photograph 46 of whole centrifuged egg, parthenogenetic.

PHOTOGRAPHS 59, 60. Parthenogenetic white half blastulae, amoeboid, with irregular nuclei. Four hours after activation. Cf. with Photograph 52, fertilized white halves.

PHOTOGRAPHS 61, 62. Blastulae both ciliate and amoeboid. Twenty-four hours after activation. Cf. Photograph 62 with Photograph 53, fertilized white half.

PLATE IV



Photographs 47–62

were taken at short intervals; after two hours the egg ended up in a two-cell stage, much as it was in the beginning. The meaning of this is not known, but perhaps it points to a similar condition, with regard to the cell surface, for amoeboid motion and cell division.

Yellow Half-egg, Parthenogenetic (Parthenogenetic Merogone)

(Photographs 85–96)

The yellow half-eggs, containing no nucleus or polar spindle, may be activated artificially by KCl. These are the parthenogenetic merogones. After activation, a thick fluted membrane is lifted off exactly like that of the normal fertilized egg, and quite characteristic of this

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PLATE V

Yellow half-eggs, fertilized. (Fertilized merogones)

PHOTOGRAPH 63. Sperm nucleus in yellow half-egg. Two hours after fertilization.

PHOTOGRAPH 64. Same egg with two nuclei, a few minutes later.

PHOTOGRAPH 65. Another yellow half-egg with several nuclei but no cell division. Five hours after fertilization.

PHOTOGRAPH 66. Two-cell stage; cells unequal.

PHOTOGRAPH 67. Typical two-cell stage. Two hours after fertilization.

PHOTOGRAPHS 68–75. Successive photographs of the same egg showing cleavage and fusion of blastomeres, and amoeboid character.

PHOTOGRAPH 68. Two equal cells. Two hours after fertilization.

PHOTOGRAPH 69. Five minutes later, 4 equal cells.

PHOTOGRAPH 70. Ten minutes later. Blastomeres begin to fuse.

PHOTOGRAPH 71. Three minutes later. Possibly polar lobe.

PHOTOGRAPH 72. One minute later. Three cells; two have fused.

PHOTOGRAPH 73. Six minutes later. Four (or 5) separate cells again.

PHOTOGRAPH 74. Five minutes later. Fusion of blastomeres, and egg becoming amoeboid. Compare with Photograph 45, of the whole centrifuged egg, parthenogenetic.

PHOTOGRAPH 75. One-half hour later, 3 hours after fertilization. Amoeboid. Compare with Photograph 46, of whole centrifuged egg, parthenogenetic; and with Photograph 58 of parthenogenetic white half and with Photograph 96 of parthenogenetic merogone.

PHOTOGRAPHS 76–84. Successive photographs at short intervals showing amoeboid character of a two-cell stage of the fertilized yellow half-egg.

PHOTOGRAPH 76. Two-cell stage, almost equal blastomeres. Fertilized at 9.30 A.M., photographed at 10.50 A.M.

PHOTOGRAPH 77, at 11.35 A.M.

PHOTOGRAPH 78, at 11.40 A.M.

PHOTOGRAPH 79, at 11.50 A.M.

PHOTOGRAPH 80, at 12 noon.

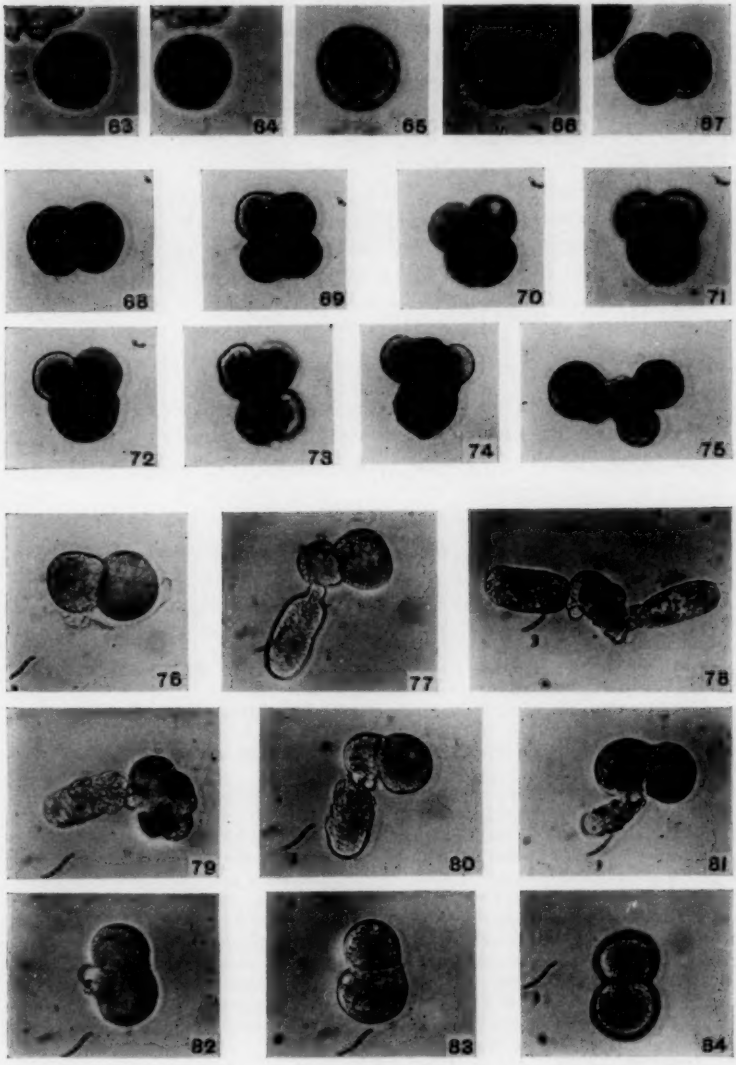
PHOTOGRAPH 81, at 12.10 P.M.

PHOTOGRAPH 82, at 12.20 P.M.

PHOTOGRAPH 83, at 12.21 P.M., two-cell stage, slightly unequal.

PHOTOGRAPH 84, at 1.10 P.M., two cells almost equal and quite similar to the original, Photograph 76. Note large nucleus.

PLATE V



Photographs 63–84

particular egg (Photograph 85, cf. Photographs 19, 21). They then usually become amoeboid and develop no further, though they may live for several days. There is no definite polar lobe, but amoeboid processes sometimes simulate it. Some cases of first cleavage have been observed, however, and these were of both the equal type and the unequal type with varying degrees of inequality (Photographs 86–90); these also became amoeboid and there was no further development (Photographs 91–93). The amoeboid activity of the parthenogenetic merogone is shown in a series of photographs taken at intervals of a few minutes (Photographs 94–96). The parthenogenetic merogone, then, resembles the fertilized merogone in its tendency to become amoeboid and in its limited cleavage, though the fertilized merogone goes perhaps one step further in development. (Compare Photographs 86–96 with Photographs 66–84).

Discussion

A study of the *Chaetopterus* egg has shown that the non-nucleate fraction of the egg of another type of animal than the sea urchin can be activated artificially. The parthenogenetic merogone of *Chaetopterus*

PLATE VI

Yellow half-eggs, parthenogenetic. (Parthenogenetic merogones)

PHOTOGRAPH 85. Yellow half, parthenogenetic, with characteristic fluted fertilization membrane. One hour after activation. At right upper corner is part of a whole cell, also with fluted membrane. Compare also with Photograph 19, of the normal whole egg.

PHOTOGRAPH 86. Two-cell stage, unequal. Two hours after activation. Compare with Photograph 66, of fertilized merogone.

PHOTOGRAPH 87. Typical two-cell stage. Two hours after activation. Compare with Photograph 67, of fertilized merogone.

PHOTOGRAPH 88. Cleaving into two almost equal cells. Five hours after activation.

PHOTOGRAPH 89. Two equal cells. Six hours after activation. Compare with Photograph 68 of fertilized merogone.

PHOTOGRAPH 90. Two equal cells. Five hours after activation. Compare with Photograph 76. Clear protrusion looks like a polar body.

PHOTOGRAPHS 91, 92, 93. Amoeboid forms of older parthenogenetic merogones. Twenty-four hours after activation. Compare with Photograph 46 of whole centrifuged egg, parthenogenetic, and with Photograph 75, of fertilized merogone.

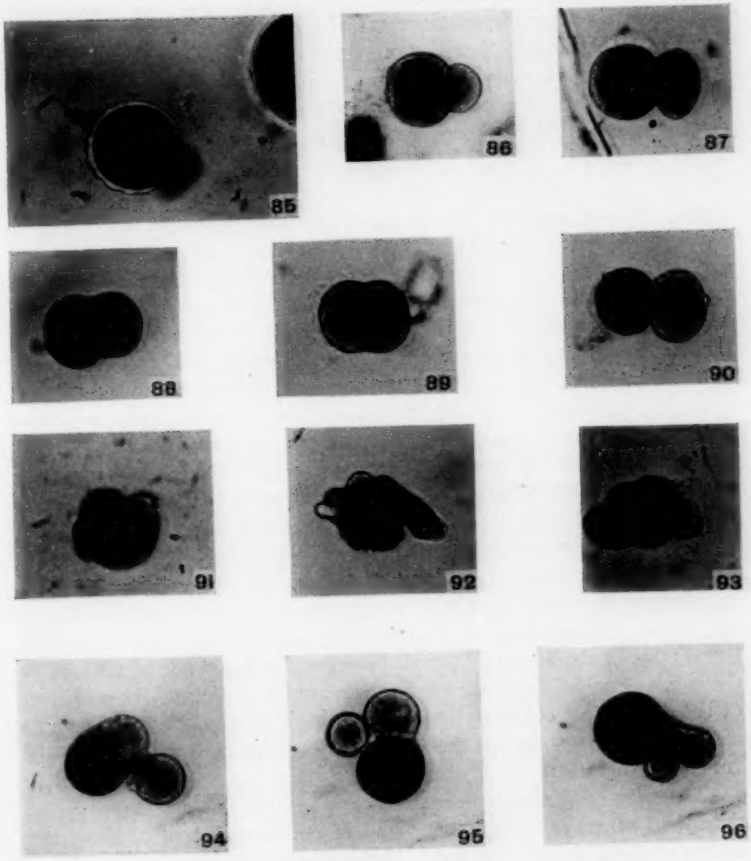
PHOTOGRAPHS 94–96. Successive photographs of the same parthenogenetic yellow half-egg, taken at very short intervals to show amoeboid character. Eighteen hours after activation. Compare with Photographs 76–84 of the fertilized merogone. Also compare Photograph 96 with Photograph 75 of the fertilized merogone, and with Photograph 46 of the parthenogenetic whole egg, and with Photograph 58 of the parthenogenetic white half.

PHOTOGRAPH 94. Activated at 5 P.M. July 21, photographed at 11 A.M., July 22.

PHOTOGRAPH 95. At 11.02 A.M.

PHOTOGRAPH 96. At 11.03 A.M.

PLATE VI



Photographs 85–96

goes through only one cleavage and thus does not develop as far as that of the sea urchin. This is not due, however, to the lack of a nucleus, since the same fraction fertilized does not develop much further. The unfertilized *Chaetopterus* egg is perhaps more highly organized than the unfertilized sea urchin egg.

The parthenogenetic development of the normal *Chaetopterus* egg is peculiar in that usually there is no orderly cleavage with the accompanying nuclear divisions, but instead, amoeboid forms with irregular cells and large nuclei. It is not surprising, therefore, that there is no orderly development of the parthenogenetic merogone, and that it likewise, becomes amoeboid. It may be that the concentration of heavy granules into this half of the egg is a factor also in preventing further development. This is suggested by the experiments of Whitaker and Morgan (1930), who obtained polar and anti-polar halves of the *Chaetopterus* egg by cutting with a needle. These halves have, of course, a more even distribution of granules than the halves obtained by centrifuging. They found that after fertilization, both of the halves would cleave unequally like the whole egg, and that the anti-polar half would form a polar lobe; there were certain irregularities, however. But the anti-polar half obtained by cutting develops more normally, at least in the early stages, than the anti-polar half obtained by centrifuging. It is of interest, too, that the parthenogenetic merogone of the *Sphaerechinus granularis* egg, whose stratification is so similar to that of *Chaetopterus* (Photographs 4 and 5) also becomes amoeboid and does not cleave regularly; the parthenogenetic centrifuged whole egg of this form likewise becomes amoeboid (see Photographs 53–56 of my 1938 paper).

The amoeboid activity which is characteristic of the parthenogenetic egg or its fraction both in *Chaetopterus* and *Sphaerechinus,* may be largely due to the action of the parthenogenetic agent on the surface of the egg. Amoeboid activity may be another expression of the condition of the cell surface characteristic of cleavage just as gelation seems to be characteristic of some of the interior cytoplasm at this time, in the formation of asters.

That the early development is dependent on the cytoplasm rather than the nucleus is brought out again in the development of the white halves of the *Chaetopterus* egg. These, whether fertilized or parthenogenetic, may cleave according to the peculiar and characteristic annelid pattern. It seems remarkable that these fractions, and even very much smaller fragments, according to Lillie and Wilson, may cleave just like the whole egg. This certainly leads one to the conclusion that it is the matrix or clear substance in the egg, rather than nuclei or visible granules that is important in early development.

Attention may be called to the frequent occurrence of equal cells in the first cleavage instead of the typical unequal cells. In the present experiments, there is a tendency to equal division in parthenogenetic whole eggs, both normal and centrifuged and both in the white and yellow halves both fertilized and parthenogenetic. Other observers have noted the same thing in certain of these cases. In the *Cumingia* egg, I found that pressure on the egg after fertilization had the same effect (Browne, 1910) and similar results have been obtained by Tyler (1930) for the *Chaetopterus* egg; he found also that cold, heat, ultra-violet rays and anaerobiosis would produce the same effect. One would assume that an unequal division of the two cells is a more specialized form, and that whatever mechanism is responsible for it, is put out of gear by certain experimental conditions. It seems to be due, at least in many cases, to an action especially on the surface of the cell, rather than on the interior.

Summary

1. The unfertilized *Chaetopterus* egg (94 μ) may be stratified and broken into unequal halves of a uniform size by centrifugal force. The nucleate white halves (83 μ) contain oil, clear layer, mitochondria and a little yolk. The non-nucleate yellow halves (64 μ) contain only yolk.

2. The centrifuged whole egg may develop, both fertilized and parthenogenetic, similarly to the normal uncentrifuged egg. Certain peculiarities have been noted.

3. The white halves, both fertilized and parthenogenetic, may cleave according to the typical annelid pattern. But there is a tendency toward equal first cleavage and amoeboid form and later, development with irregular cells and large nuclei; the blastulae tend to fuse together; these peculiarities are much more pronounced in the parthenogenetic than in the fertilized white halves.

4. The yellow halves, both fertilized (= fertilized merogone) and parthenogenetic (= parthenogenetic merogone) usually lift off a characteristic fluted fertilization membrane and pass through only one cleavage and become markedly amoeboid.

5. Early development without nuclei (parthenogenetic merogony) is established for the annelid, *Chaetopterus,* in addition to the five species of echinoderms previously studied. The development of the parthenogenetic merogones of *Chaetopterus* does not go as far as that of the sea urchin.

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CHEMICAL MEDIATION IN CRUSTACEANS. III. ACETYLCHOLINE AND AUTOTOMY IN PETROLISTHES ARMATUS (GIBBES)

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Acetylcholine has been shown to be present in nervous tissues of crustaceans in considerable amounts (Welsh, 1938; Smith, 1939) and to have an excitatory action on the decapod heart (Welsh, 1939 *a* and *b*), but, thus far, its action elsewhere in crustaceans has not been demonstrated (see Katz, 1936, regarding acetylcholine and crustacean skeletal muscle).

Autotomy, or the casting of legs by crustaceans, is a well-known phenomenon resulting from a unisegmental, reflex stimulation of the autotomizer muscle of a leg (Wood and Wood, 1932). Hence central transmission at synapses, peripheral transmission at myoneural junctions, and conduction along fibers of the reflex pathway must occur in this process. If acetylcholine is normally involved in the transmission of nerve impulses in the autotomy reflex, it should be possible to obtain evidence by appropriate injections of acetylcholine and of drugs which are known to affect its rate of destruction and its action. That stimulating and inhibiting substances may play a part in autotomy in *Porcellana* and *Uca* was suggested by Hoadley (1934, 1937). Abramowitz and Abramowitz (1938), while testing a series of drugs for their effects on the chromatophores of *Uca*, observed that the injection of acetylcholine caused autotomy.

The present paper records observations made on autotomy in an anomuran of Bermuda. The experimental work was done at the Bermuda Biological Station and was aided by grants from the Milton and Porter Funds of Harvard University.

Materials and Methods

The animal used was *Petrolisthes armatus* (Gibbes), of the family Porcellanidae. The members of this family are said to autotomize with the greatest ease and after the least stimulus of any of the decapod crustaceans (Wood and Wood, 1932). *P. armatus* occurs in consid-

erable abundance under stones between tide levels in Bermuda. They were captured and handled with care in order to prevent autotomy, and experiments were performed, whenever possible, on animals which had been in the laboratory less than twenty-four hours.

Since *Petrolisthes* may be caused to autotomize a leg by grasping it gently with forceps, this was the method of stimulation employed. Individuals were placed in small containers filled with sea water. Each leg was seized, held for approximately one second, and, if not dropped, was then released. *Petrolisthes* has three pairs of legs which are developed for walking, and a pair of chelae, so a series of eight stimuli could be given the animal without repeated stimulation of those appendages which failed to autotomize on the first trial. Legs were grasped in random order and the order was varied from animal to animal.

After obtaining results on one hundred normal animals to serve as controls, fifty animals were injected at the base of one of the last walking legs with 0.05 cc. of a perfusion fluid prepared according to Pantin (1934). As the average body volume of the animals used was only 0.3 cc., this amount of injected fluid caused a considerable dilution of the blood, but this dilution and the act of injection had no evident effect on autotomy. The drugs employed were always given in the volume of fluid used in the injected controls. These drugs were acetylcholine chloride (Hoffman-LaRoche), eserine (physostigmine) sulphate (Merck), atropine sulphate (Merck), and adrenalin chloride (Parke-Davis). Fresh solutions of these substances were prepared daily and the necessary dilutions were made just before using. This is especially important in the case of acetylcholine which is rapidly hydrolyzed in an alkaline solution.

Results

Control Experiments.—The results obtained on uninjected control animals are given in Fig. 1. The first leg [1] stimulated by grasping was dropped by 54 of the 100 animals, or it may be said that there was 54 per cent autotomy. The second, third and fourth legs stimulated showed increasing tendency to autotomize up to 85 per cent. Then an abrupt drop in the number of autotomies appeared until the eighth, or last leg, autotomized in only 21 per cent of the trials. This increasing tendency to autotomize up to a certain point, followed by a decreasing tendency, is not due to individual differences in the autotomizing mechanisms of different legs, for with such differences alone a varying, random order of stimulation would give a straight line parallel to the abscissa. It

[1] No distinction will be made between walking legs and chelae and both types of appendages will be spoken of as legs.

can be explained, however, by assuming that each stimulus to a leg, whether the leg is dropped or not, affects the tendency of the other legs to autotomize. Up to the fourth stimulus the effect is excitatory and after the fourth stimulus it is inhibitory.

Fifty *Petrolisthes* injected with 0.05 cc. of "crab Ringer" and stimulated ten to fifteen minutes after injection autotomized in a manner similar to the uninjected controls (Fig. 1).

The line which is drawn through the data shown in Fig. 1 may be called the normal curve of autotomy for *Petrolisthes,* when stimulation

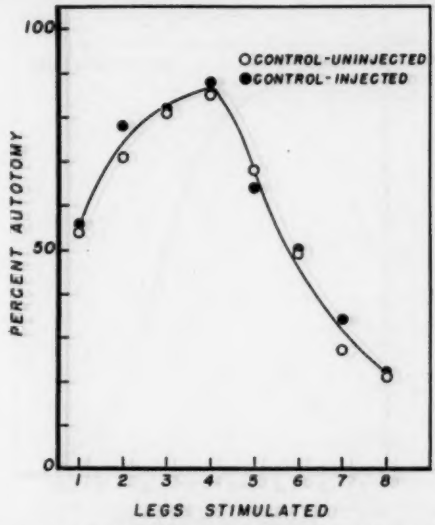

FIG. 1. Percentage of legs dropped by 100 normal animals when each leg was stimulated once, and the order of stimulation was varied from one animal to the next, is shown by the open circles. Results obtained on 50 animals injected with 0.05 cc. of "crab Ringer" are shown by the closed circles. The line drawn through the data has been called the "normal curve" of autotomy.

is as described. An obvious change in the "normal curve" produced by the injection of some substance would give an indication of the effect of that substance on the process of autotomy.

Acetylcholine.—The injection of various amounts of acetylcholine in preliminary tests indicated that 25 gamma of acetylcholine [2] was intermediate between a lethal dose and one whose effects had largely disappeared between the time of injection and the time of stimulation. In

[2] The amount of substance injected will be expressed as the weight of the salt even though the salt is not indicated. The volume of fluid was always 0.05 cc.

most cases larger amounts caused a number of legs to be dropped during the injection process, or shortly after the injection was made. The legs dropped were almost always on the side on which the injections were made.[3] In 20 per cent of the animals the injection of 25 gamma of acetylcholine caused one to three legs to be dropped without further stimulation.

Fifty animals, each of which was injected with 25 gamma of acetylcholine and its legs stimulated approximately five minutes after injection, yielded results which are shown in Fig. 2. The first leg to be grasped

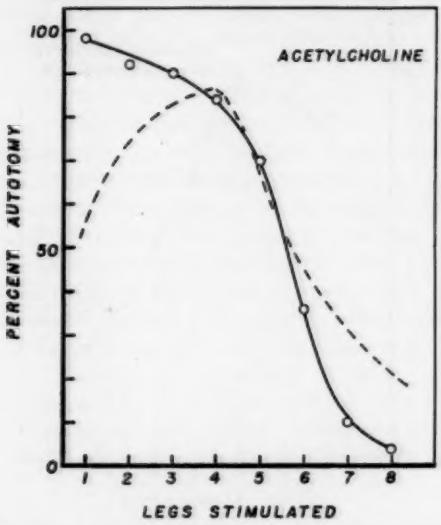

FIG. 2. Percentage autotomy in 50 animals each of which received 25 gamma of acetylcholine chloride five minutes before stimulation of the legs. The dashed line in this and the following figures is the "normal curve" of autotomy.

was dropped in 98 per cent of all the animals, but immediately an "inhibitory" phase appeared and the entire curve resembles the inhibitory part of the "normal curve." It is apparent that the injection of acetylcholine markedly affects the autotomizing process and, at this concentration, eliminates the period of increasing tendency to autotomize.

Eserine.—Petrolisthes was found to be remarkably sensitive to eserine. The injection of 0.5 gamma or more caused, after a delay of one

[3] The injection of 100 gamma of acetylcholine into each of six *Pachygrapsus transversus* (Gibbes), a rock crab, caused the autotomy of three to five legs, on the side of the injection, during the injection process. In two cases all of the legs on the opposite side were autotomized within a few seconds after injection.

to two minutes, extreme activity followed by tetanic convulsions and death. The injection of 0.25 gamma caused greatly increased general activity and uncoördinated movements followed by sluggishness, failure to autotomize, and the death of some animals. A dose of 0.1 gamma caused increased activity for a few minutes after injection, but following this the reactions of the majority of the animals appeared normal. This amount was administered to fifty animals in groups of ten, and ten to fifteen minutes later their legs were stimulated as were those of the controls. The results may be seen in Fig. 3. The eserine curve is quite

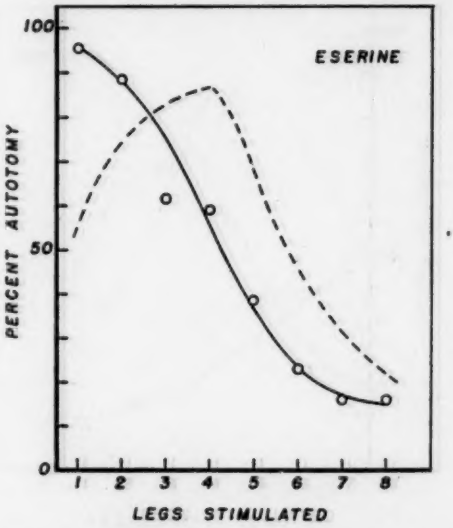

FIG. 3. Percentage autotomy in 50 animals injected with 0.1 gamma of eserine sulphate and their legs stimulated ten to fifteen minutes after injection.

similar to the one obtained for acetylcholine, except that it is slightly lower and shifted somewhat to the left due to a lower percentage of autotomies in all except the last two legs to be stimulated. The important points, however, are the absence of a period of increasing tendency to autotomize, and the large number of autotomies (95 per cent) of first legs to be stimulated.

Atropine.—Relatively large doses of atropine had to be injected before definite effects were obtained. Amounts above 100 gamma caused marked sluggishness and prevented all autotomies during a period of fifteen minutes after injection. A dose of 100 gamma resulted in de-

creased general activity for a period of five minutes after the injection but ten minutes later the animals appeared normal, hence this was selected as the dose to be employed. Eighty animals were injected in lots of ten, and ten minutes later their legs were stimulated. Fifteen animals failed to drop any legs and in the calculations of percentage of autotomies these were omitted. The remaining sixty-five animals showed a marked reduction in the total number of legs dropped, as may be seen in Fig. 4. Only 46 per cent dropped a leg at the first stimulus and this was followed by a decline in the number of autotomies with

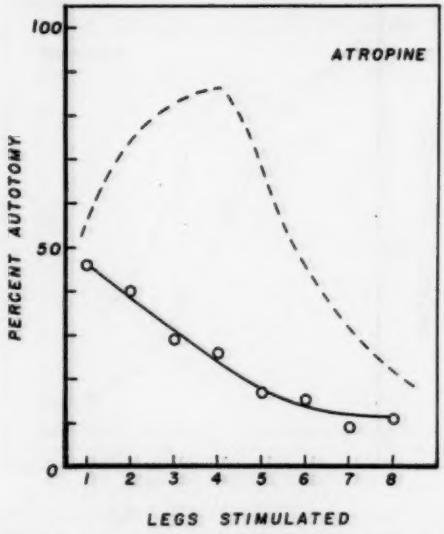

FIG. 4. Percentage autotomy in 65 animals injected with 100 gamma of atropine sulphate and their legs stimulated ten minutes after injection.

subsequent stimuli. Thus atropine inhibits autotomy more or less completely, depending on the amount injected.

Adrenalin.—Adrenalin (adrenine) is known to have an excitatory action on the decapod heart (Bain, 1929; Welsh, 1939*a* and *b*), therefore it seemed desirable to test its effects on autotomy in *Petrolisthes.* Adrenalin chloride was injected in amounts varying from 1 gamma to 50 gamma per animal. A dose of 50 gamma produced marked inactivity, uncoördinated movements and occasionally death. A dose of 1 gamma had no noticeable effects, either on general behavior, or on autotomy. Fifty animals injected with 2.5 gamma of adrenaline and stimulated

ten to fifteen minutes later gave results which are shown in Fig. 5. The shape of the curve is not very different from the "normal curve," but its displacement indicates that adrenalin, in this concentration, has a slight inhibitory effect over the entire series of legs stimulated.

Autotomy in Males and Females.—The following observations have only an indirect bearing on the general question of chemical mediation and autotomy, but seem worthy of mention. Evidence presented by Hoadley (1934; 1937) indicated that in *Uca* and *Porcellana,* females without eggs and males tended to autotomize more readily than did fe-

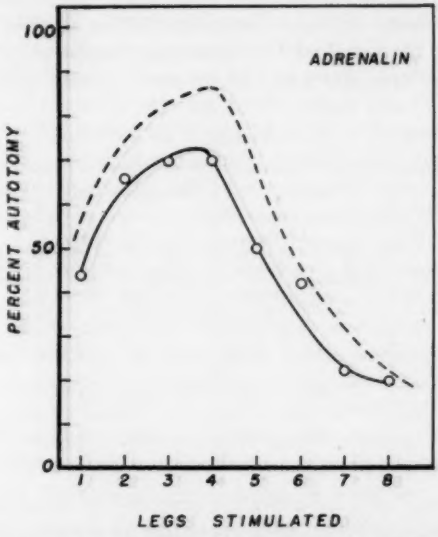

FIG. 5. Percentage autotomy in 50 animals injected with 2.5 gamma of adrenalin chloride and their legs stimulated ten to fifteen minutes after injection.

males with eggs. Our data on control animals, including one group of one hundred animals which have not been mentioned previously, were examined to see if *Petrolisthes* showed similar differences. The great majority of the females were carrying eggs at the time our observations were made; hence the results for the small group not carrying eggs are of little significance. Table I includes the data from 249 animals. It is apparent from these data that the tendency to autotomize in *Petrolisthes* is not modified by the carrying of eggs. However, this does not preclude the possibility of there being such a modification in other groups of crustaceans.

Discussion

It has been shown (1) that stimulation of the legs of *Petrolisthes armatus* produces changes in the animal which result in an increasing percentage of autotomies for the first four legs followed by a progressive decrease in the number of autotomies for the remaining legs; (2) that the injection of acetylcholine facilitates or causes autotomy; (3) that eserine, which prevents the enzymic hydrolysis of acetylcholine, produces certain effects similar to those produced by injected acetylcholine; (4) that atropine, which prevents the muscarin-like action of acetylcholine, causes a partial or complete reduction in the number of autotomies. This evidence indicates that acetylcholine normally acts in the autotomy reflex of *Petrolisthes*. Interesting questions are: (1) where and in what way does this action occur, and (2) why does the stimulation and autotomy of a leg affect the tendency of remaining legs to be dropped? These and other questions cannot be definitely answered with the evidence at hand but certain suggestions, to be tested by further experiments, may be offered.

TABLE I

	Males		Females with eggs		Females without eggs	
	No. of animals	Average no. legs dropped per animal	No. of animals	Average no. legs dropped per animal	No. of animals	Average no. legs dropped per animal
Regular order of stimulation	52	4.50	45	4.38	3	5.0
Random order of stimulation	49	4.44	48	4.95	3	5.0
Injected controls	23	4.74	22	5.13	4	5.0

Although the ventral ganglia of *Petrolisthes* are fused into a single mass, as in the Brachyura, there is an anatomical arrangement according to a segmental pattern. Stimulation of a leg by pinching excites sensory fibers to the ventral ganglion supplying that segment. Internuncial neurones carry the impulses to the motor fibers which innervate the autotomizer muscle of that leg. Since it is extremely rare for any leg to be dropped other than the one being stimulated, it is apparent that any impulses going out from a ganglion to other ganglia must ordinarily be below threshold for the autotomizer muscles of other legs. However, sub-threshold impulses might produce facilitation, as has been shown by

Pantin (1934; 1936*a* and *b*) for leg muscles of *Carcinus,* and the facilitating process might be the release of acetylcholine at motor nerve endings. There is good evidence that acetylcholine is present in crustacean blood in considerable amounts (Smith, 1939). Its accumulation, as a result of successive stimuli, might account for the regular increase in the number of autotomies of the first four legs stimulated in the control animals. Why, with subsequent stimuli, there should then be a regular decrease in the number of autotomies of the remaining legs is not readily explainable. In accounting for certain phenomena associated with Wedensky inhibition and fatigue in vertebrate muscle, Rosenblueth and Morison (1937) have postulated that high concentrations of acetylcholine are paralytic. Possibly after the fourth leg of *Petrolisthes* is stimulated the acetylcholine concentration in the blood reaches a paralytic range and inhibition then sets in. However, if this were the case, on the basis of the control data one would expect that 85 per cent autotomies would be the maximum obtainable before the paralytic threshold of acetylcholine were reached; instead, injections of acetylcholine and eserine may result in as high as 98 per cent autotomies.

Rosenblueth and Morison (1937) have suggested that in high frequency stimulation of vertebrate nerve the quantal yield of acetylcholine, per impulse, is greatly reduced as stimulation is continued. A similar situation may occur in *Petrolisthes* as successive volleys of impulses traverse the reflex pathways. Taking into consideration the rate of production and the rate of destruction of the acetylcholine it follows that its concentration in the general circulation would rise to a maximum followed by a sharp decline as stimulation is continued. If, then, the degree of facilitation of autotomy is directly dependent on the concentration of acetylcholine in the region of the autotomizer muscles, one would expect precisely the type of curves obtained from the control animals and from those injected with acetylcholine. Additional data must be obtained, however, before this explanation is entirely acceptable.

What appears to be a clearly demonstrable rôle of acetylcholine in autotomy in crustaceans is of interest in itself but of greater interest is the evidence that acetylcholine may, after all, be the transmitter or mediator substance between motor nerves and skeletal muscle of crustaceans.

Summary

1. A single stimulus to each of the walking legs and chelae of the anomuran, *Petrolisthes armatus,* was found to cause, in most animals, the autotomy of four or more of these eight appendages. The appendages least often dropped were the first, sixth, seventh, and eighth of a series, when the order of stimulation was a random one. A regular

increasing tendency to autotomize in the first four legs was followed by a decreasing tendency in the remainder.

2. Injection of acetylcholine in relatively high concentrations caused the autotomy of one to several legs without further stimulation. A concentration was found which only occasionally caused autotomy when injected, but which facilitated the autotomy of the first three legs to be stimulated and eliminated, thereby, the period of increasing tendency to autotomize.

3. The injection of eserine caused a marked increase in general activity and a relatively small amount (0.1 gamma per animal) facilitated the autotomy of the first and second legs. The injection of eserine never caused legs to be dropped unless they were stimulated, otherwise its effects on autotomy were very similar to those produced by acetylcholine.

4. Atropine caused a general lowering of excitability and in relatively high concentrations completely prevented autotomy.

5. The injection of adrenalin was followed by a reduction in the percentage of autotomies of each of the eight legs but the phases of increasing and decreasing "excitability" remained unchanged.

6. No differences, correlated with sex or the bearing of eggs, were found in the average number of autotomies per individual.

7. The evidence obtained has been interpreted as indicating that acetylcholine normally plays a rôle in the autotomy reflex of *Petrolisthes* probably by acting as a mediator of impulses between the motor nerve and autotomizer muscle of a leg.

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SEXUAL PHASES IN TERRESTRIAL NEMERTEANS

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There has been much confusion in the literature regarding the sexuality of the various species of terrestrial nemerteans, due partly to the small number of individuals that have been available for study and partly to the fact that in some cases only the older and larger individuals have been collected. Since some of the species have a distinct tendency toward protandry and some are irregularly hermaphroditic, the successive stages in the life history are necessary in order to determine the potential sexuality of the individual.

Particularly confusing have been the accounts of the sexual conditions in *Geonemertes palaensis*, a species found on Pelew (Palao), Samoa and several other tropical Pacific islands. A collection consisting of 21 representatives of this species recently submitted to the writer for study included individuals representing such a series of sexual phases as will give a presumably reliable clue to their sequence. This occasion has also been taken to re-examine the consecutive sexual phases of the Bermuda land nemertean (*G. agricola*) as described many years ago (Coe, 1904).

The 12 apparently valid species of terrestrial nemerteans which have been described up to the present time all belong to the genus *Geonemertes*. Three of these, *G. australiensis* Dendy, *G. hillii* Hett, and *G. rodericana* (Gull.) are described as of separate sexes and oviparous, with the males usually smaller than the females. Three others, *G. dendyi* Dakin, *G. graffi* Bürger and *G. novae-zelandiae* Dendy have been thought to be of separate sexes, although no males have yet been found. One species, *G. spirospermia* Darbishire, is known only from a single male and another, *G. caeca* Darbishire, from a single female. *G. chalicophora* Graff was first described as hermaphroditic, although Böhmig (1898) found only females. *G. palaensis* Semper is hermaphroditic, as originally described and as found by von Kennel (1878), but only ovaries were present in the single specimen studied by Schröder (1918) as well as in the five investigated by Hett (1928). *G. arboricola* Pun-

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nett is likewise hermaphroditic and, as pointed out by Hett (1928), resembles *G. palaensis* so closely as to suggest specific identity.

All the above are known or assumed to be oviparous but the land nemertean of Bermuda, *G. agricola* (Will. Suhm), has been shown to be irregularly protandric, hermaphroditic and viviparous (Coe, 1904). The egg clusters of *G. australiensis* have been described by Dendy (1893) and those of *G. dendyi* by Waterston and Quick (1937). Also one of the specimens of *G. palaensis* studied by Hett (1928) had an egg cluster attached to the proboscis.

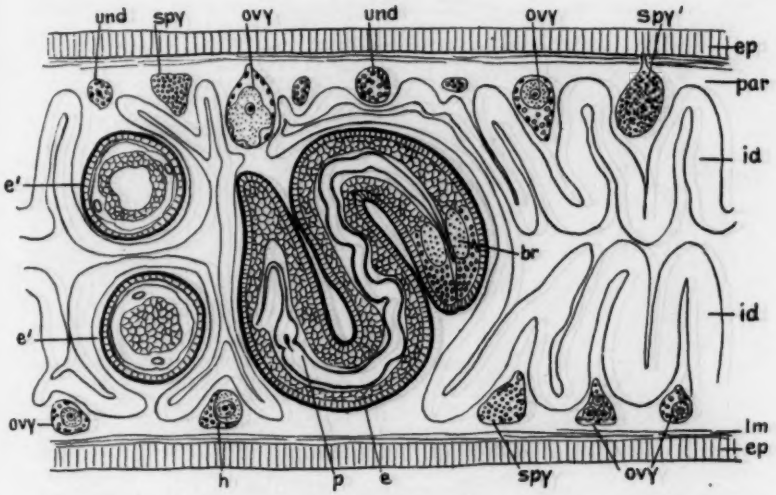

FIG. 1. *Geonemertes agricola.* Portion of horizontal section of body dorsal to nerve cords, showing sections of two embryos (*e*, *e'*) nearly ready for birth. Small gonads representing young ovaries (*ovy*), young spermaries (*spy*), spermaries with ripe spermatozoa (*spy'*), gonads of the hermaphroditic type (*h*), as well as others still remaining undifferentiated (*und*) are already present in anticipation of the following sexual phases. Other letters represent: *br*, brain of embryo; *ep*, epithelium of body wall; *id*, intestinal diverticula; *lm*, longitudinal musculature of body wall; *p*, proboscis of embryo, *par*, parenchyma. Slightly diagrammatic.

SEXUAL PHASES IN G. AGRICOLA

The study of more than a hundred individuals representing all ages of *G. agricola* shows that the young worms are usually protandric, since at first sexual maturity they have their bodies distended with spermaries. After the discharge of the sperm or sometimes earlier, ovaries begin to develop and the ovocytes slowly grow to maturity in the first female

phase. Before the eggs are ready for fertilization, however, other gonads in the same individual may develop rapidly into spermaries, and some of these may form functional spermatozoa in time to fertilize the eggs of the same individual or of another individual which may be in a similar female phase.

Shortly after fertilization the oviducts are closed and the eggs undergo development. In some individuals only a few embryos are formed (Fig. 1) but in others the numbers are so great that the mother's body

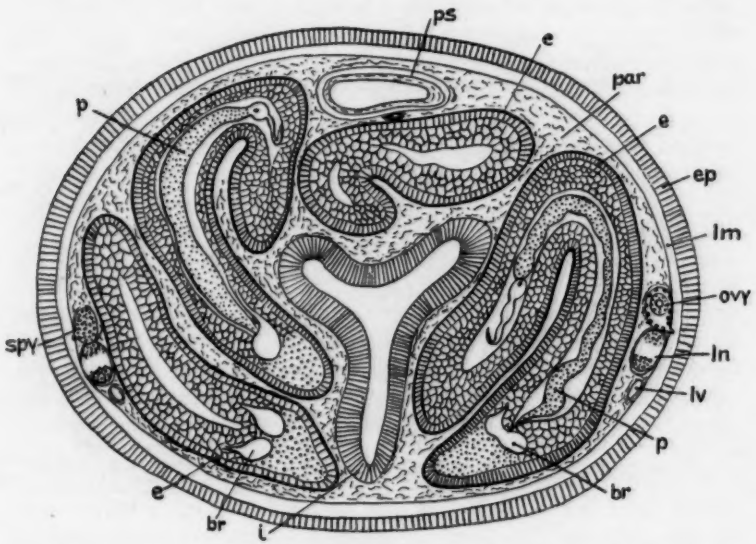

FIG. 2. *Geonemertes agricola.* Transverse section of body distended with embryos of which four have been cut in this section; showing also small ovary (*ovy*) and small spermary (*spy*); *i*, intestine; *ps*, proboscis sheath; other letters as in Figure 1. Slightly diagrammatic.

becomes enormously distended (Fig. 2). After all the organ systems of the adult except the reproductive organs have been acquired the young worm forces its way out of the mother's body by rupturing her body walls.

Even while embryos remain in the body, preparations are in progress for the succeeding sexual phases, both spermaries and ovaries continuing to be differentiated (Fig. 1).

After the discharge of the embryos such spermaries as have not previously been active now become functional as the second male phase.

This is later followed by the second female phase, with some new spermaries becoming functional when the ova are fully ripe.

The primary male phase is thus followed by a female phase which often changes to a functional hermaphroditic phase before the ova are ready for fertilization. Then follows a series of male and female (or hermaphroditic) phases, probably with seasonal interruptions of considerable duration, throughout the lifetime of the individual. There is much individual variation, however, in regard to the relative preponderance of male and female characteristics and time of maturity of each type of sexual products, since some individuals in the female or hermaphroditic phase have many large ovaries and few spermaries; others have

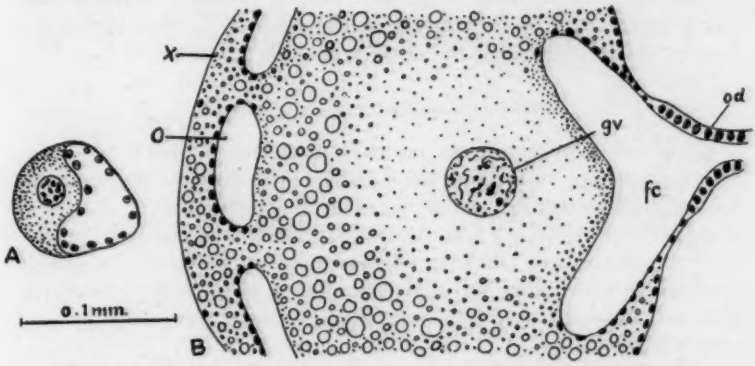

FIG. 3. *Geonemertes agricola*. *A*, young ovary with single ovocyte and associated follicle cells lining ovarian cavity. *B*, nearly mature ovary, showing portion of the single ovum with part of original ovarian cavity converted into a fertilization chamber (*fc*) continuous with the oviduct (*od*); *x*, peripheral cytoplasm; *o*, extensions of ovarian cavity lined peripherally with yolk-forming follicle cells; *gv*, germinal vesicle surrounded by yolk-free cytoplasm. Scale indicates magnification.

a true female phase with no spermaries at the time. Variations in the proportion of primary gonads which are to become differentiated into the two types of sexual glands and differences in the time of their development would account for the diversity of sexual conditions observed.

Differentiation of Gonads

It has been previously reported (Coe, 1904) that the gonads, all of which are primarily ambisexual in nature, become differentiated into three types before maturity. In those which are to form ovaries the ovocyte increases rapidly in size, first by assimilating smaller potential ovocytes and spermatogenic cells (if present) and later by yolk-forma-

tion through the activities of the follicle cells in the peripheral layer of cytoplasm (Fig. 3) as described more fully in the account of ovogenesis in *G. palaensis* in the following section of this report. In the spermaries the potential ovocytes are inhibited in their growth by the multiplication of spermatogonia.

Relatively few of the gonads retain their primary ambisexual character to maturity by the harmonious development of both types of gametes. These few hermaphroditic gonads apparently discharge their spermatozoa previous to yolk-formation by the ovocyte. In other cases the ovocyte in such gonads undergoes fragmentation and cytolysis following the discharge of the sperm.

The ripe ova are from 0.35–0.45 mm. in diameter, or from one-fourth to one-half as great as the diameter of the entire body of the worm. Development is of the direct type (Coe, 1904).

Sexual Phases in G. palaensis

This species appears to be normally oviparous as evidenced by the finding of an egg cluster by Hett (1928). The material now available for study shows that the sexual phases are similar to those reported previously for *G. agricola* (Coe, 1904) and summarized above. The primary functional male phase is followed similarly by successive female (potentially hermaphroditic) and male phases, the extent of hermaphroditism depending on the time of development of the spermaries and their relative number.

Of the 21 specimens in this collection 2 were in the functional male phase at the time of collection, with ripe spermaries and immature ovaries; 3 were functionally female at the time of collection, with ovaries showing various stages of ovogenesis but no spermaries; 8 were unripe sexually with small ovaries and undeveloped spermaries; and 8 were functional hermaphrodites with large ova and ripe or nearly ripe spermaries.

The potentially hermaphroditic phases may in certain individuals or at certain periods in the sexual sequence be predominantly male or predominantly female or exclusively functional as one sex or the other. A greater number of individuals will, however, be found in the female phase than in the male phase of sexuality, since the time required for the development of the ovaries is much greater than for the spermaries. Consequently, if the collectors had obtained only such of these specimens as were in the exclusively female phase at the time of collection, it would have been concluded that the species must be of separate sexes. It is suspected that this may be the case with some of the other species which

are known from only one or a few specimens and in which neither protandry nor hermaphroditism has been observed.

The males or the primary male-phase individuals, whichever they may be, are smaller than the functional females and consequently less

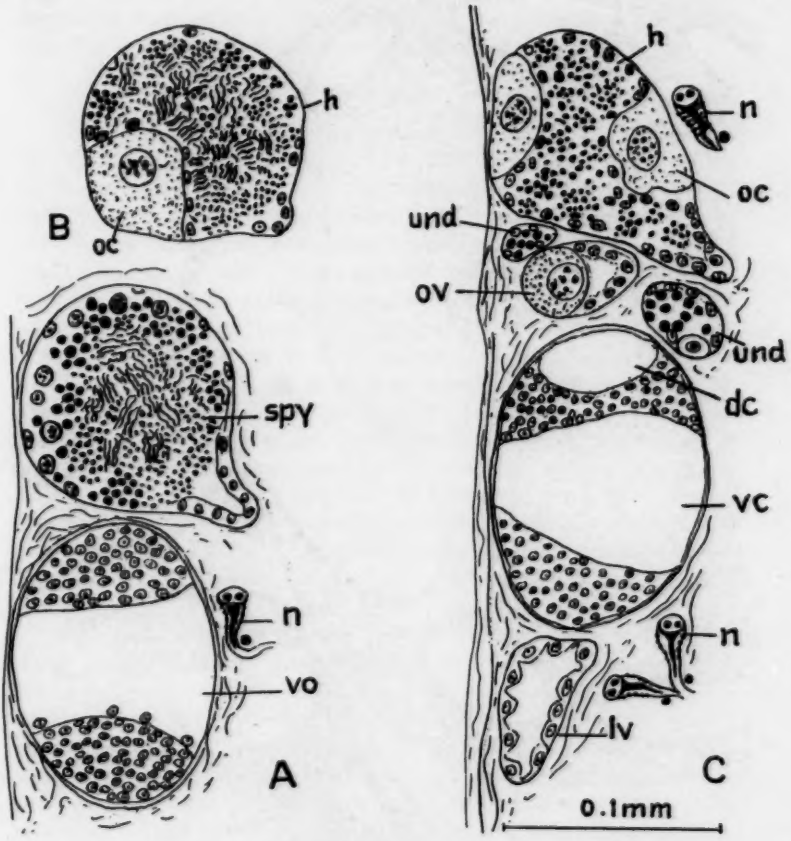

FIG. 4. *Geonemertes agricola*. *A*, transverse section of lateral nerve cord (*vo*) and adjacent spermary (*spy*) from individual containing fully grown embryos; *n*, a single flame cell of nephridial system. *B*, hermaphroditic gonad with single ovocyte (*oc*) from same individual. *C*, *Geonemertes palaensis*. Portion of transverse section of body, showing hermaphroditic gonad (*h*) with spermatids and two small ovocytes (*oc*), one small ovary (*ov*) with ovocyte and follicle cells, also two undifferentiated gonads (*und*). Other letters as in Figure 5. Scale beneath drawing shows magnification.

likely to be found by collectors. In *G. dendyi*, for example, no male or male-phase individual has yet been reported although females have been found by Dakin (1915) in the natural habit of the species, in Aus-

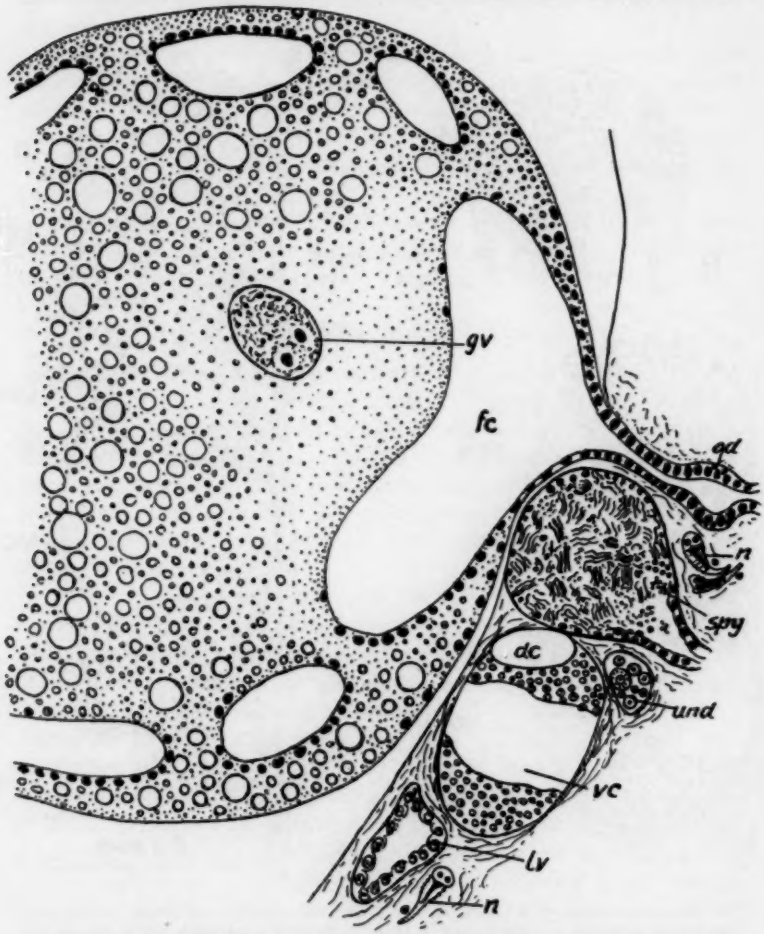

FIG. 5. *Geonemertes palaensis*. Portion of transverse section of body, showing fully ripe spermary (*spy*) and mature ovum ready for fertilization, also a small, undifferentiated gonad (*und*). Other letters indicate: *fc*, portion of original ovarian cavity, now converted into the fertilization chamber continuous with the oviduct (*od*); *gv*, germinal vesicle; *dc* and *vc*, dorsal and ventral cores of lateral nerve cord; *lv*, lateral blood vessel; *n*, flame cells of nephridial system. Same magnification as Fig. 4.

tralia, and by Stammer (1934) in Germany and Waterston and Quick (1937) in Wales, into which countries it has been introduced. Stammer has suggested the possibility that all the terrestrial species are protandric hermaphrodites. Such proves to be the condition in the two species which form the basis of the present paper but the extent to which the suggestion may apply to other species can be determined only after their life histories have been more completely investigated.

Ovogenesis

The young ovary usually has a single ovocyte on the wall nearest the center of the body, with a number of small follicle cells lining the ovarian cavity (Figs. 4, 5). As the ovocyte increases in size it becomes partially separated from the ovarian wall by wide spaces continuous with the ovarian cavity, eventually remaining attached to the wall by broad pseudopodia only. These pseudopodia then spread out and flow together distally to form a continuous cytoplasmic lining on the ovarian wall. This peripheral cytoplasm becomes widely separated from the main body of the ovocyte by these extensions of the ovarian cavity but remains united with it by large protoplasmic connections (Fig. 5). The surface of the ovum is thereby greatly increased.

Meanwhile the follicle cells have multiplied rapidly and spread as a continuous layer of tissue covering the peripheral cytoplasm and now function in the formation of yolk. As rapidly as the yolk is formed it is carried by cytoplasmic streaming into the main portion of the rapidly growing ovocyte. With the completion of yolk-formation, the peripheral cytoplasm is drawn into the ovocyte which then assumes a nearly spherical form.

Before the egg is mature the follicle cells lining the walls of the distal ovarian chamber form a continuous, epithelial-like layer which grows distally through the connective tissue and muscular layers of the body wall to form the oviduct (Figs. 4, 5). When the ovum is fully ripe the oviduct is completed through the surface epithelium of the body and provides an opening through which sperm may enter and through which, in the oviparous species, the egg will be discharged later. The remaining ovarian cavity provides a fertilization chamber through which the sperm passes before entering the yolk-free animal pole of the ovum (Fig. 5). The presence of two mature ova in a single ovary is very unusual in *G. palaensis* but occurs occasionally in *G. agricola*. In the young ovaries, however, two or more ova are of common occurrence (Fig. 4) but all except one of these are ingested or cytolysed during ovogenesis. The yolk, however, is formed more largely by the activities of the follicle cells than by the ingestion of the smaller ovocytes.

General Discussion

Development of Gonads

In the hermaphroditic species three types of gonads are often present in the same individual: (*a*) spermaries, (*b*) hermaphroditic gonads which have retained their early ambisexual character and contain both sperm-forming cells and immature ovocytes, and (*c*) ovaries. The relative proportion of each of these three types at each sexual phase is highly variable. Except in young individuals the ovaries are much more numerous than either of the other types. Indeed, there are often several hundred times as many ovaries as spermaries and in some cases the worm passes through a strictly female phase. In other individuals there may be a few spermaries at either end of the body or they may be scattered between the ovaries. It must be remembered, however, that this condition applies to the individual at the time of observation or the time of collection and does not indicate the sexuality which has obtained at an earlier period of life or might have followed later if the animal had survived.

The hermaphroditic gonad may occasionally function first as a spermary and later as an ovary but more often the ovocytes present undergo cytolysis before the sperm are fully mature. Otherwise each gonad functions but once, its place being taken by a younger one following the discharge of the gametes, or of the embryo in the viviparous species, as *G. agricola*. New gonads are formed throughout life from groups of germinal cells situated along the dorsal borders of the nerve cords (Figs. 4, 5). It is conceivable that the sexual differentiation of the gonad may depend upon the nutritive conditions of that region of the body at the time of differentiation. Presumably when closely crowded or when originating in an unusual position on the distal side of the nerve cord, as well as when rapidly growing ovocytes or embryos are present nearby, the gonad becomes differentiated into a spermary. When a spermary develops in the same interdiverticular space as an ovary the former usually lies nearest the nerve cord.

Fertilization

In nearly all the species in which living individuals have been available for study the worms have been observed to collect together in groups with the bodies of two or more individuals placed side by side, accompanied by the secretion of considerable mucus. If two or more of these individuals are provided with ripe sexual products when thus in contact the sperm cells from one individual may be readily transferred to the fertilization chambers of another which may contain

mature ova and open oviducts. Internal insemination occurs in all terrestrial species so far as known but is not confined to that group, since it is also characteristic of the fresh-water nemerteans and some of the marine species.

In *G. australiensis,* in which the sexes are separate, such copulation and the transfer of sperm from the small male to the larger female has been observed (Dendy, 1893). The sperm thus transferred to the ovaries may remain functional for several days or perhaps weeks, ready to enter the egg in each ovary as soon as it has become sufficiently mature. Although the male in *C. dendyi* has not yet been discovered, Waterston and Quick (1937) reported that several of the females collected by them laid clusters of fertilized eggs a few days after capture.

In the hermaphroditic species the young, male-phase individual presumably may thus transfer his sperm to either an individual functioning at the time as a female or to an hermaphrodite with both ripe eggs and sperm; likewise two hermaphrodites may transfer sperm mutually or an hermaphrodite may inseminate a functional female. It seems quite possible that in some cases individuals of all these sexual phases may be grouped together at the same time. Microscopic sections sometimes show the presence of several spermatozoa in a fertilization chamber.

In terrestrial nemerteans insemination thus precedes ovulation if two individuals with suitable sexual products come in contact and this is the case with a few marine and fresh-water forms. In the great majority of nemerteans, however, the eggs are fertilized at the time of ovulation or in some cases, as in *Cerebratulus,* vast numbers of minute ova are set free in the water with the possibility of fertlization by sperm originating from a male which may be at least several meters distant.

There appears to be no fixed limit to the number of sexual phases which an individual may experience during its lifetime, since even in the largest worms bearing ripe ova or embryos, according to the species, there are always present many newly-formed, undifferentiated gonads in the parenchyma adjacent to the dorsal sides of the nerve cords (Figs. 1, 4). Recuperation periods, some of which may be of a seasonal nature, may, presumably, long delay the completion of any of these phases.

Self-fertilization

In the hermaphroditic species, as *G. agricola* and *G. palaensis,* spermaries with fully functional sperm are frequently present in some part of the body and sometimes immediately adjacent at the time when the ova are ready for fertilization. It would appear that in isolated individuals the conditions are highly favorable for self-fertilization, although direct evidence of such an occurrence is not available. It is well-

known, however, that in some hermaphroditic nemerteans, as the freshwater *Prostoma,* self-fertilization takes place frequently.

It is remarkable that among the 12 known species of terrestrial nemerteans, all of which belong to a single genus, there should be so much similarity in morphological and ecological characteristics and so great a diversity in the physiology of reproduction. Some of the species are hermaphroditic and others presumably of separate sexes; most of them are known or believed to be oviparous but at least one species is regularly viviparous.

Viviparity, protandry and hermaphroditism occur also in a few species of marine nemerteans belonging to different groups, and in some species of *Prostoma, Carcinonemertes* and *Prosorhochmus* which are normally oviparous, with internal fertilization, a few of the fertilized ova may be retained in the mother's body until the embryo is far advanced. Since in *Geonemertes* the same type of fertilization exists and at least one of the species is regularly viviparous it seems quite possible that occasional viviparity will later be found in some of the little-known species now thought to be exclusively oviparous.

Summary

1. A series of 21 specimens of a species of terrestrial nemertean, *Geonemertes palaensis,* from tropical Pacific islands includes successive stages in the development of the gonads which show that this species is normally protandric and irregularly hermaphroditic, as previously described for *G. agricola.*

2. The primary male phase of the young individual is followed by a female phase which, however, may become functionally hermaphroditic in some individuals before the ova are fully ripe by the precocious development of some of the spermaries of the normally succeeding male phase. Opportunity is thereby offered for self-fertilization.

3. The extent to which the female phase toward its close is accompanied by the development of spermaries is highly variable, some individuals having in this phase only a few small spermaries and others many larger ones.

4. This sequence of alternating male and female (or hermaphroditic) sexual phases presumably continues throughout the lifetime of the individual.

5. *Geonemertes palaensis* is oviparous, while the terrestrial nemertean of Bermuda (*G. agricola*) is viviparous. The sexual phases, however, are similar in both species.

6. No direct evidence is available as to whether the great variability in the relative proportions of male and female gonads in different indi-

viduals following the initial male phase in both these species is due to physiological conditions imposed by environmental influences or to slight differences in the hereditary sexual endowment. Presumably both these factors may be associated.

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MORTALITY OF THE COD EGG IN RELATION TO TEMPERATURE

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The general fact that the rate of development of fish eggs becomes greater as the temperature is increased is well known, but knowledge of the relative mortality of the eggs at different temperatures is poor. In nature, the extent of mortality of the egg is particularly important in the case of marine fish which produce pelagic eggs because such eggs may be carried by currents from the spawning grounds into regions of different temperature. The location in which the majority of the eggs will ultimately hatch depends not only upon the rate of development but more upon the relative mortality at various temperatures encountered. (*Cf.* Bigelow, 1926; Fish, 1927.) Conversely, in cases where developing eggs or larvae are taken in plankton hauls, the site at which the eggs were spawned may be traced provided the rates of development and mortality are known and the velocity and direction of the current and temperature of the water can be ascertained. (*Cf.* Walford, 1938; Jacobsen and Johansen, 1908.)

The experiments described in this paper were undertaken to test the extent of mortality in the cod egg at various temperatures and to determine the highest temperature at which development is possible for this species.

Dannevig (1894), who made the first comprehensive attempt to find the temperature range for the cod, was able to hatch eggs from 0° to 14° C. Johansen and Krogh (1914), the most recent investigators in this field, have confirmed this pioneer work. Although chiefly interested in the temperature coefficient, they carried out experiments to establish the upper temperature limit, which they placed at 10.2° C., as eggs hatched at this temperature but failed to do so at 12°, 13°, 16.5° and 20° C.

Investigators who have been more directly concerned with mortality in fish development are Drouin de Bouville (1908), Hein (1907, 1911), Hata (1927), and Rollefsen (1932). Hein subjected trout eggs to adverse conditions at various times after fertilization and demonstrated

[1] The author wishes gratefully to acknowledge the advice and encouragement of Dr. George L. Clarke of Harvard University.

that variations in mortality occurred in relation to the length of time development had proceeded. Rollefsen has confirmed these results, particularly for the early stages of development, and has correlated the decrease in susceptibility to mechanical shock, with the closing of the blastopore.

Procedure

The cod egg was chosen for these experiments because it normally floats at the surface of the water while alive and sinks when dead or dying, thus presenting a clear-cut criterion of death. Since the chorion is transparent, an unhampered view of the embryo is afforded and consequently the stage of development may be easily determined. Material was readily obtained through the courtesy of the Gloucester Station of the United States Bureau of Fisheries.

Eggs were obtained from cod taken in Ipswich Bay, Massachusetts, on March 31, 1937. The surface temperature was 2.5° C. The eggs were transported in insulated jugs at 2.5–3° C., to the laboratory where they were apportioned into beakers at the different constant temperatures. Eight hours had elapsed since fertilization but due to the low temperature the eggs were only just past first cleavage. (Plate I, Fig. 2.)

The 500-cc. beakers used were open at the top, and only 200 cc. of aerated sea water were introduced in order to provide a relatively large surface area for gas exchange. The beakers were placed on racks in five constant temperature tanks maintained to within 0.3° C. at 6, 8, 10, 12, and 14° C. respectively, by means of thermo-regulators. The first four tanks were placed in a cold room at 2° C. and each appropriately heated. The fifth tank was kept in a laboratory at room temperature and cooled to the desired temperature by means of a Kelvinator refrigerating unit.

The sea water, which had a salinity of 32.31‰, had been obtained at Nahant, Massachusetts, six months previously and allowed to stand. During this period all organic matter had decomposed and fouling of the water during the experiment was reduced to a minimum. Although this decomposition process reduced the oxygen content so that thorough aeration of the sea water was necessary before use, it was not necessary to aerate the jars containing the eggs during the experiment.

The eggs were distributed among twenty beakers and four beakers were placed in each of the five tanks. Each day the eggs were transferred to fresh beakers containing water that had been prepared in advance and adjusted to the proper temperature. Transfer was accomplished by means of a scoop made of fine mesh nickel screening.

The eggs which sank to the bottom—and hence were judged to be dead—were removed daily from each jar by pipette, counted and preserved. The fixative used was Stockard's solution [2] which turns the embryo white and leaves the yolk clear. This facilitates the determination of the degree of development in preserved material.

In many cases the eggs were examined before preservation and there was no doubt that they were either dead or dying.

The live eggs in two of the beakers (jars *A* and *B*) in each tank were sampled daily in small numbers to determine the stage of development. They were examined before preservation and the stage carefully noted according to the plan presented in Plates I and II. In every sample the eggs were all found to be very near the same stage. In a few cases in which the stage of a small number departed noticeably from that of the majority, the embryos were obviously monsters or aborted forms whose development could not have proceeded much further. These abnormal forms were encountered more frequently during the early days of development than later.

This daily sampling of jars *A* and *B* resulted in a continued reduction of the total numbers of eggs. Since it was feared that this change in concentration might influence the mortality, parallel tests without sampling were made in the third and fourth beakers in each tank (jars *MA* and *MB*). Only dead eggs were removed in the case of these two beakers. However, the mortalities which resulted in the jars that were sampled turned out to be substantially the same as those obtained for jars that were not sampled.

Rate of Development and Drift at Sea

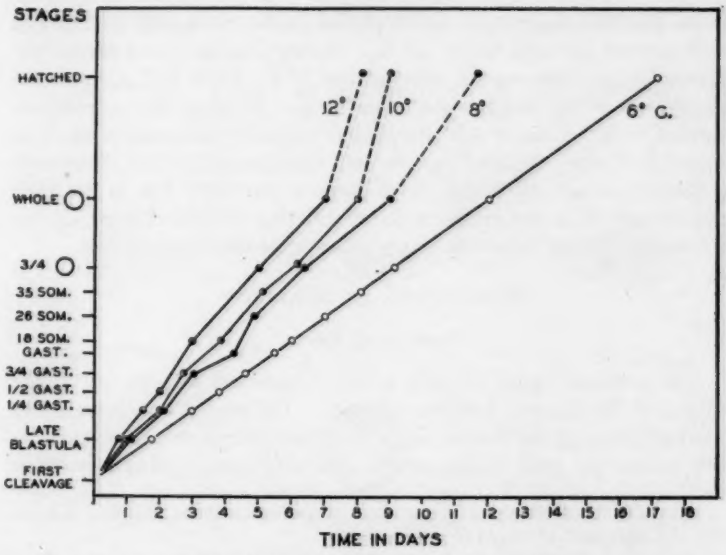
The considerable lapse in time between spawning and hatching for the various temperatures tested may be appreciated from the curves plotted in Fig. 1. The tests run at 14° showed no survival after 24 hours and a subsequent test at 13° also failed. Hence, 12° C. was concluded to be very near the upper limit of temperature for the development of the cod egg. The low survival at this temperature and previous work of Dannevig (1894) and Johansen and Krogh (1914) are in accord with this view.

The young fry of the cod are believed to take to the bottom 7 days after hatching according to McIntosh and Masterman (1897). It is of interest to calculate the point to which Ipswich Bay cod eggs will drift and to ascertain whether the young will find themselves in a suitable locality when they are ready to take to the bottom. According to Fish

[2] Stockard's solution consists of 5 parts 40 per cent formalin, 6 parts glycerin, 4 parts glacial acetic acid, and 85 parts distilled water.

(1927) most of the eggs produced in Ipswich Bay do not drift into Massachusetts Bay but are carried off to the southward. These eggs may be supposed to drift at a rate similar to those leaving Massachusetts Bay, which were shown by Fish to move about 3 or 4 miles per day. At 6° development to hatching required 17 days. With 7 days added to reach the bottom, the eggs would drift 72 to 96 miles.

It is probable that the temperature at which the Ipswich Bay eggs develop is considerably lower than this. Bigelow and Welsh (1925)



TEXT FIG. 1. The development of the cod egg at four different constant temperatures. Abscissa: Time in days. Ordinate: Stage of development determined by the 6° C. which is arbitrarily plotted as a straight line. The curves for the other temperatures plotted against the stages so determined.

state: "The chief production of cod eggs is during the cold months, on the Ipswich grounds, for example, ripe fish are taken when the bottom water is still as warm as 6.6 to 7.7° C. in early September, but they appear in great numbers in temperatures of 5° to 6° C. in January, and as the breeding progresses, the temperature falls, spawning being at its height in the minimum temperature of the year (March), that is at 0.5° to 3.0° C." Poulsen (1931), McKenzie (1934), and others agree that cod may spawn at temperatures ranging from 8° down to 0° C. At a temperature of 3° C. hatching occurs in 23 days according to

Dannevig (1894). With 7 days added for larvae to take to the bottom, at a maximum of 4 miles per day the egg would travel 120 miles from its spawning ground. In the present case such a journey would carry the egg to the northwestern edge of Georges Bank. This accords well with Fish (1927) that the eggs spawned in inshore areas such as Ipswich Bay take to the bottom on Georges Bank.

We may next inquire whether the temperature in this region during the critical months ever becomes sufficiently high to result in serious mortality to the cod eggs. Bigelow (1927) has shown for the Gulf of Maine that the temperatures which prevail during the spring months and early summer are well below 12° C. During February and March the highest surface temperature recorded was 5° C., while during April, the temperature at the surface was below 6° C. In May, the waters have warmed to 10° C. in a few places, but the temperature does not rise above 12° C. until the latter part of July and during August. Inasmuch as this is well after the eggs have hatched, mortality due to too high temperatures is not normally a factor limiting the distribution of the developing cod egg from the Ipswich Bay spawning grounds.

Observations on Mortality

Sources of Error

The principal source of error in this experiment was the use of the sinking of the egg as a criterion of death. On several occasions it was noted in sampling the floating eggs that there were some eggs, particularly among the early stages, which were only masses of cells without

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Stages in the development of the cod. Magnification 30 ×. Figures marked with (*) were used as stages of development.

Fig. 1. Protoplasmic cap, prior to first cleavage. Drawn from material preserved 15 minutes after fertilization.

Fig. 2*. Two-cell stage. Ten hours after fertilization at 0°–3° C. Stage at distribution of eggs.

Fig. 3. Eight-cell stage, polar view. Twenty-eight hours after fertilization at 0°–3° C.

Fig. 4. Early blastula, 52 hours after fertilization at 0°–3° C.

Fig. 5. Middle blastula. Seventy-six hours after fertilization at 0°–3° C. Twenty-four hours after Fig. 2 at 6° C.

Fig. 6*. Late blastula. One hundred hours after fertilization at 0° C. Twenty-four hours after Fig. 2 at 10° C.

Fig. 7. Early gastrula. Forty-eight hours after Fig. 2 at 6° C.

Fig. 7*a*. Ibid. Polar view.

Fig. 8*. One-quarter gastrula. Fifty-two hours after Fig. 2 at 6° C.

Fig. 9*. One-half gastrula. Seventy-three hours after Fig. 2 at 6° C.

Fig. 9*a*. Ibid. Eccentric polar view.

Fig. 10*. Embryo beyond the three-quarters gastrulation stage; 120 hours after Fig. 2 at 6° C. First specks of pigment appearing.

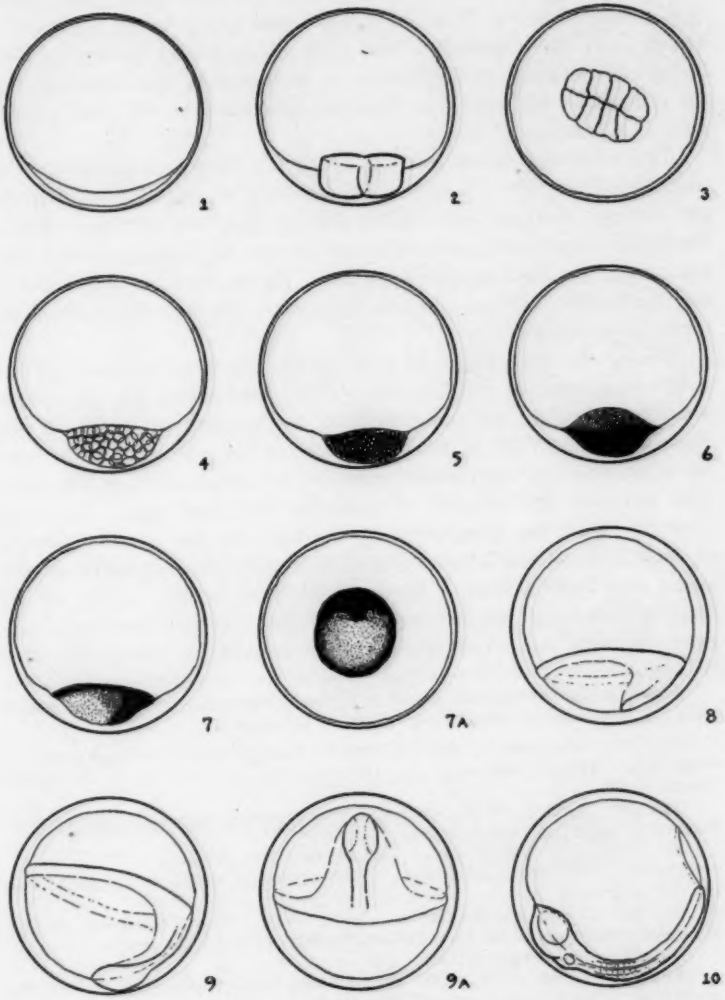

PLATE I

any morphological features to determine the stage. Brice (1898) states that floating eggs are not necessarily alive, for unfertilized and injured eggs usually float for 18 to 36 hours before going to the bottom. In dealing with daily mortality, one must recognize the possibility of a lag of this duration. Observations on such aborted eggs indicated that the number at any time was negligible in relation to the total number which sank during 24 hours.

That some eggs which sink may be alive is likewise a possibility. In fact, McIntosh (1893) states that in certain of his experiments with the cod, the eggs retained their vitality although they did not float. But in the present experiment, examination of the non-floating eggs showed that less than 1 per cent appeared normal. Furthermore, it was observed that death soon followed when the eggs sank, although development did continue for a short time.

During the later stages of development this criterion becomes less certain, for Jacobsen and Johansen (1908) have shown that the specific gravity of the egg becomes greater as development proceeds. It was observed that at 6° C. all the eggs floated at the surface until the tenth day after which the eggs became scattered in various layers in the water. This increased the difficulty of removing only dead eggs. Therefore from the tenth day opaqueness of the egg was used as the criterion of death. Since only about 2 per cent of eggs removed contained embryos with beating hearts, important numbers of live eggs were not being removed with the dead eggs. Although a certain number of dead eggs which had not yet become opaque were probably missed, the effect

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Stages in the development of the cod. Magnification 30 ×. Figures marked with (*) were used as stages of development for graphs.

FIG. 11*. Just prior to whole gastrula or final closing of the blastopore; 126 hours after two-cell stage at 6° C. (Plate I, Fig. 2.) Embryo of 11 somites. Apolar view.

FIG. 12*. Embryo of 18 somites; 144 hours after two-cell stage at 6° C. Pigment definitely appearing.

FIG. 12*a*. Ibid. Apolar view. Pectoral limb buds forming.

FIG. 13*. Embryo of 35 somites. Tail twisting to the right; 192 hours after two-cell stage at 6° C.

FIG. 14*. Three-quarters circle. Apolar view. Heart beginning to beat. Muscular movements of the tail; 216 hours after two-cell stage at 6° C.

FIG. 14*a*. Ibid. Lateral view.

FIG. 15*. Whole circle. Tail approximates head. Upper part of eye well pigmented; 288 hours after two-cell stage at 6° C.

FIG. 16. Just prior to hatching. Pigment localized into definite bands. Eye well pigmented; 396 hours after the two-cell stage at 6° C.

FIG. 17. Lateral view of head of Fig. 16.

FIG. 18. Larval cod about three days after hatching. Floats with yolk-sac uppermost during first two days after hatching at 6° C.

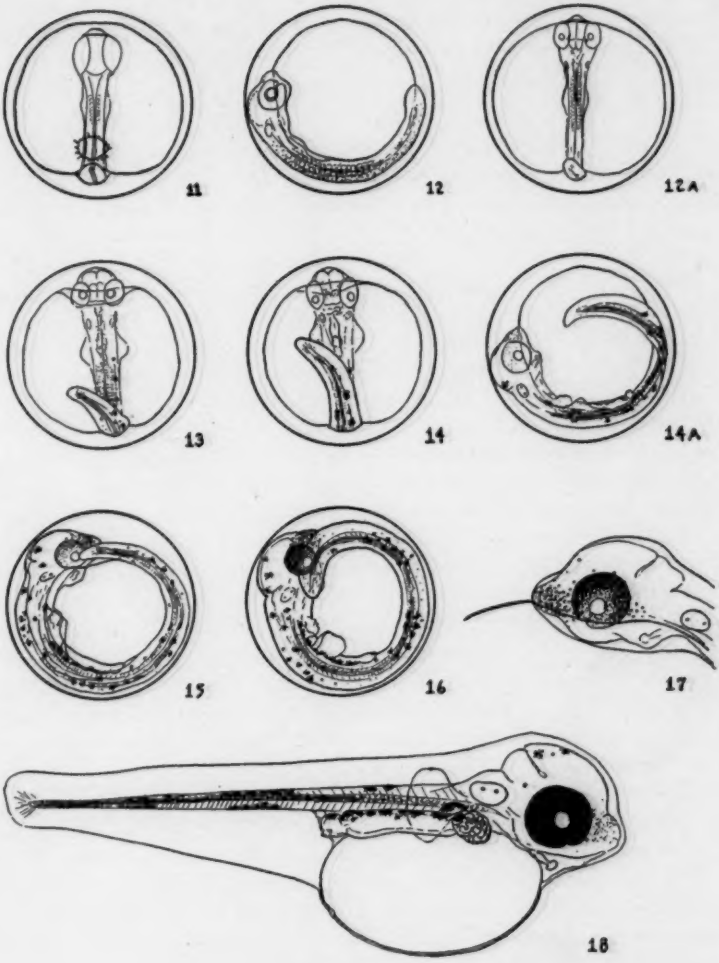


PLATE II

of this procedure merely introduced a delay in the time when those eggs would be counted as dead. The high mortality which occurred in these experiments does not interfere with an interpretation of the results, for the interest lies not in the number of deaths but in the distribution of mortality in relation to the stage of development.

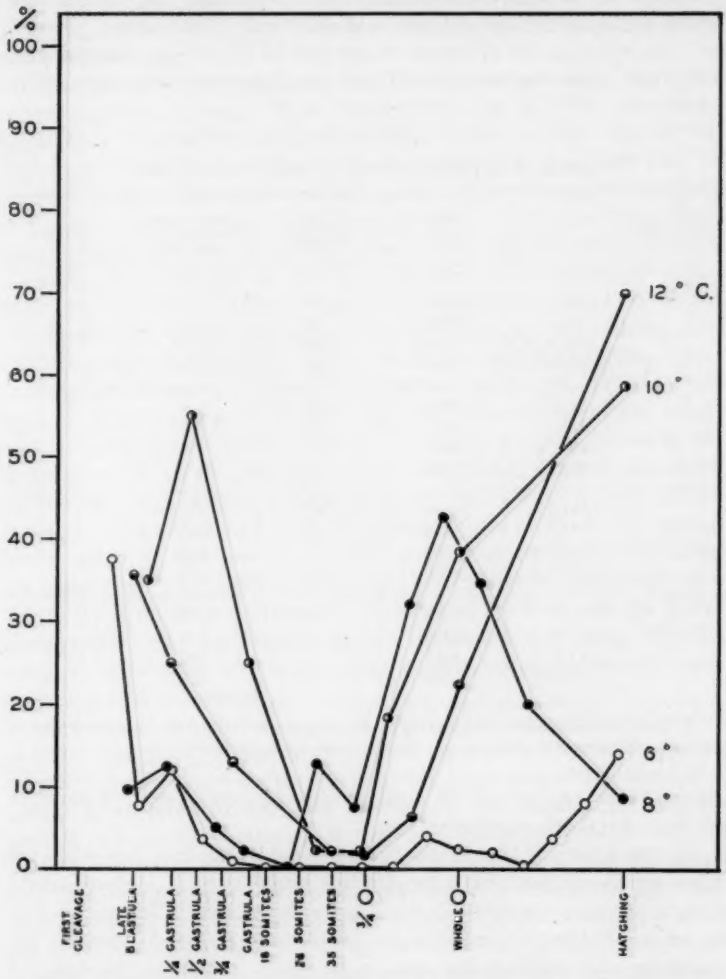
Results of Experiments

The time required for the eggs to reach various stages at different temperatures may be observed in Fig. 1, in which the data obtained from daily sampling have been plotted following the method used by Worley (1933) for the mackerel. Those stages in development of the cod which have been used for this purpose are indicated in the legends of Plates I and II. The stages plotted are not of equal duration, as they were chosen on the basis of certainty of recognition. They have been spaced on the graph in such a way as to make the 6° C. development approximate a straight line. The development of the eggs at the other temperatures was then plotted against these same ordinates.

Confusion has existed in the past over the use of "hatching" as a stage in development because the times when the first and last egg hatch are ordinarily observed directly whereas in the case of the other stages, the samples of eggs withdrawn for microscopic examination allow only the modes of the stage to be determined. Obviously, if the time for each of the earlier stages is taken as the mode for the whole population, then the time designated for the hatching stage should be the point at which the largest number of eggs hatch. Merriman (1935), in his work on the cut-throat trout, however, has taken the time to "first hatching" as one stage and the "eyed-out" condition as another stage. This procedure is fallacious unless the moment when the first embryo entered the eyed-out stage was observed. But even if this were done, the method gives a poorer measure of the rate of development for the whole population than the use of the mode.

Merriman objects to employing the middle of the hatching period as the time to "hatching" because he finds that the "*duration*" of the hatching period (i.e. the time from the hatching of the first egg to the hatching of the last) varies with the temperature in a different manner than the duration of the whole period of development. But the amount of "spread" of the eggs in any stage has no necessary connection with the changes in average time for complete development at different temperatures. Furthermore, in stages other than "hatching" the time that each egg remains in a given stage may be affected differently by temperature at the various points of development. The influence of tem-

perature on the total time for development, and on the time elapsed in each stage should therefore be carefully distinguished. In the present case the information desired is the average total time for development



TEXT FIG. 2. The percentage mortality of the cod egg in relation to stage of development. Abscissa: Stage of development. Ordinate: Percentage daily mortality. (The number of eggs that died during any day divided by the number of eggs that were alive at the beginning of that day multiplied by 100.)

involving a summation of the average times in each of several stages. Therefore, the time taken should be the time required for the "modal" or typical egg at each temperature. Worley (1933) accomplished this by means of frequency distribution. In the present experiment, the number hatching during each day was observed. The time of "hatching" was taken as the day when 50 per cent of all the eggs that hatched during the entire hatching period had completed their development in

TABLE I

Percentage of eggs hatching at different constant temperatures

Temperature	Jar	Total no. of eggs	Number hatched	Percentage hatched of total eggs	Average
°C.				*per cent*	*per cent*
6	IA	1653	10	0.6	11.2*
	IB	2059	8	0.4	
	IMA	118	20	16.9*	
	IMB	546	148	27.1*	
8	IIA	1913	84	4.3	3.3
	IIB	2975	124	4.1	
	IIMA	1811	70	3.8	
	IIMB	1509	15	1.0	
10	IIIA	1820	60	3.2	1.9
	IIIB	339	7	2.0	
	IIIMA	1638	6	0.3	
	IIIMB	1730	37	2.1	
12	IVA	2831	2	0.07	0.04
	IVB	2837	0	0.0	
	IVMA	2312	0	0.0	
	IVMB	1690	2	0.12	

* These values are not comparable to the other figures since the initial number of eggs is very low and the factor of crowding must be considered.

the egg stage,—a method not quite as accurate but more convenient in the case of a slowly developing egg.

A comparison of the curves of Fig. 1 reveals the fact that when the stages are so arranged that a straight line results at 6° C., approximately straight lines are obtained for the other temperatures. This means that the effect of different temperatures on the rate of development of the modal egg was relatively the same in all stages. At 6° C. development was almost twice as long as the development at 12°. This compares favorably with the results obtained by Dannevig (1894) and Johansen and Krogh (1914). The difference between 10° and 12° is much less

than that between 6° and 8° although the temperature change in both cases is 2° C. The explanation lies in the fact that we are dealing with the upper part of the temperature range, the optimal temperature being about 6.5° C., and as the maximum is approached there is a retardation of development. Worley (1933) has demonstrated a similar situation in the mackerel.

The percentage mortality at the different temperatures has been plotted against the stage of development in Fig. 2. The percentage mortality is obtained by dividing the total number of live eggs at the beginning of any 24-hour period into the number of eggs that died in the same period. During the hatching period all eggs which had hatched on previous days were subtracted from the total. The stages of development were interpolated from Fig. 1, and the curves presented here are the mean values for all the jars in each tank. In order to determine accurately the total number of eggs in each jar at the beginning of the experiment, the number of eggs that died daily and the number that hatched were added together. These totals are tabulated in Table I.

Special mention must be made of the 8° C. curve. The first batch of eggs all died on the first day due to a failure in the apparatus which caused a sudden rise in temperature. A second lot of eggs which had been held at 2° C. for 24 hours before being placed at 8° C. was used. At this time, they were at a stage corresponding to early blastula (Plate I, Fig. 4). The wide variation which occurred day by day in the various jars in the 8° C. tank seems to indicate an extraneous factor influencing the mortality. The consistency between the individual jars would indicate a factor common to them all. In view of the fact that the curves for the other tanks are consistent among themselves, one should view the curve of the 8° tank with reservations in considering the general implications of this experiment.

An examination of the curves in Fig. 2 of the percentage mortality at 6, 10, and 12° C. shows that there is general agreement in two respects: a decided decrease in mortality during closing of the blastopore and an increase in mortality as hatching is approached. A different way of stating this conclusion is that the susceptibility of the egg to harmful factors in the environment is least between closure of the blastopore and the first signs of muscular movement, which occurs when the embryo occupies approximately three-quarters of the circumference of the egg (Plate II, Fig. 14). The curve for 8° C. shows the same general trend but the large fluctuations appear to have no satisfactory explanation. The important feature to be noted in these curves is their similarity. There is a decline in mortality toward the same stage irrespective of the temperature. To be sure, the length of the period of

low mortality is longest at 6° C., which is near the optimum temperature, but the periods of high mortality are the same for 6° as for the other temperatures. Since greater numbers of eggs die at 12° C., the higher average position of the curve is understandable.

Rollefsen (1932) has shown that a decrease in susceptibility of cod eggs to shock caused by dropping occurred coincident with the closing of the blastopore. This he explained as an increase in the strength of the yolk covering, preventing rupture. Hein (1907, 1911) performed a series of experiments on trout eggs, subjecting them to various types of adverse conditions at different times after fertilization, and found a similar decrease in susceptibility down to closure of the blastopore and an increase just prior to hatching. My results have confirmed these earlier experiments, but it is not possible at the present state of our knowledge to make any statement concerning correlations between susceptibility and ontogenetic stage beyond the gross description of coincident states.

Summary

1. Experiments were carried out at four different temperatures in the supra-optimal range of development for the cod egg.

2. Development from the two-cell stage to 50 per cent hatched required 8.5 days at 12° C., 9 days at 10° C., 11.5 days at 8° C., and 17.2 days at 6° C.

3. At all temperatures an initial period of high mortality decreased with the closing of the blastopore and was followed by a period of low mortality until the embryo was three-quarters the circumference of the egg membranes, when the mortality steadily increased up to hatching.

4. The hatching period is discussed with reference to its use as a stage of development.

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THE TIME OF EMBRYONIC DETERMINATION OF SENSORIA AND ANTENNAL COLOR AND THEIR RELATION TO THE DETERMINATION OF WINGS, OCELLI, AND WING MUSCLE IN APHIDS[1]

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In a general analysis of the applicability of the time-of-determination theory to intermediate aphids by Stiles (1938), nothing was said regarding the color of antennae and number of sensoria except to include them in the same tables with the wing, ocellar, and wing muscle development. The purpose of this paper is to report a study of the time of embryonic segregation of the sensoria and antennal color in the aphids previously reported (Stiles, 1938).

Shull, in investigations of gamic-parthenogenetic (1933) and winged-wingless (1937) intermediate aphids, did not include individuals whose intermediacy consisted in the color of their antennae because of the apparently erratic variability of that color. Nevertheless, antennal color and sensoria number are both characters very definitely related to the winged condition of aphids, hence should receive consideration in a study of forms intermediate between winged and wingless. Because most of the intermediates of this study did not express any intermediacy in their antennae, it was difficult to relate closely the intermediacy of this organ with that of the wings, ocelli and wing muscle. No closely graded series of antennal color or sensoria number could be found; therefore, it became apparent that these characters were probably of less value for this type of investigation than the wings, ocelli and wing muscle. It is for this reason that the records are somewhat incomplete regarding the nature of the antennae in the data presented (Stiles, 1938). However, enough antennae were studied to make possible an analysis of their relation to the general problem of intermediacy.

In my previous paper the antennae of the typical wingless aphid with respect to color are rated 0 (light) and that of the winged as 4 (dark), with intermediates represented by numbers between these. While 4 to 6 sensoria in the third segment of the antenna are typical

[1] Contribution from the Department of Zoölogy, University of Michigan.

in the wingless (Fig. 1 *A*) and about 15–18 in the winged aphids (Fig. 1 *B*), it is not considered safe, due to variation, to judge less than 10 sensoria as representing certain intermediacy in an antenna, although there are doubtless some with less that should be so considered.

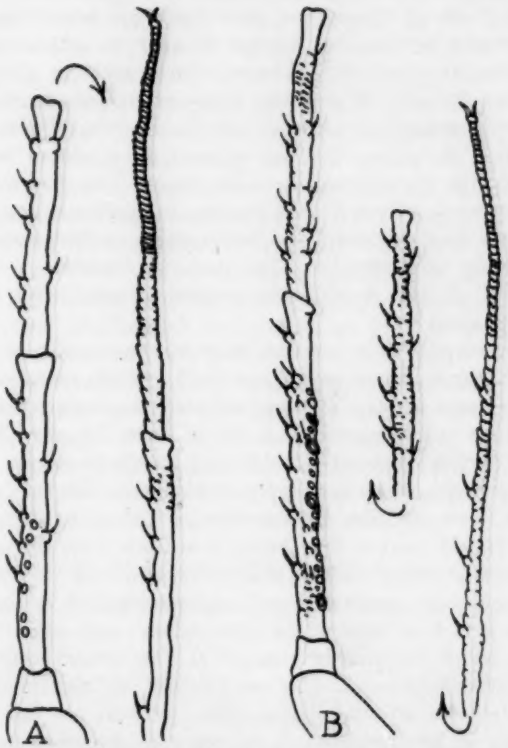


FIG. 1. Antennae of *Macrosiphum solanifolii* showing the difference in number of sensoria in typical wingless and winged forms. Sensoria are represented by the small circles on segment III. *A*, Wingless parthenogenetic female. *B*, Winged parthenogenetic female.

The data presented in Tables [2] I to IX inclusive (Stiles, 1938) show that the color of antennae and sensoria number are closely related; that is, in general a darkening of antennae is accompanied by an increase in sensoria number. The author is inclined to believe that, in a case

[2] To save republication of tables, all tables cited in this paper refer to a previous paper (Stiles, 1938).

where a slight darkening of the antennae may have been recorded with no corresponding increase in the number of sensoria, an error has been made in judging antennal color. Unless the color difference is marked, it is difficult to be sure of the antennal color. Nevertheless, records of both antennal color and sensoria number are useful inasmuch as one provides a check on the other. Since there has been found to be a close correlation between the number of sensoria and antennal color, with the number of sensoria a more reliable guide to antennal intermediacy than the color of antennae, henceforth these structures will be indicated by statements concerning only the sensoria.

With few exceptions, the data showed a correlation between the sensoria and the character of the wing muscle. An intermediate condition of sensoria, indicated by an increase in number as compared with wingless, was, in almost every such intermediate studied, associated with non-degenerate wing muscles. The one most notable exception is a case in which there is so little degeneration of muscle that degeneracy may be questioned.

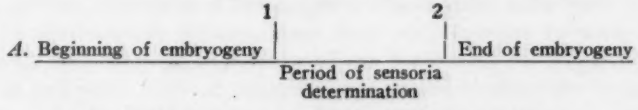
Another correlation is apparent between intermediate sensoria and quantity of muscle, for in general increased sensoria are associated with a relatively large amount of wing muscle. An intermediate of particular interest in this connection is one in Table VI, slide 1061 *e* (1), wing .5, ocellar development .94, and wing muscle development .99 with 14 and 15 sensoria, which is almost normal for the winged. It has been difficult to relate the time-of-determination theory to the number of sensoria. On the basis of this theory, if sensoria were determined very late, as might be suspected from their correlation with wing muscle, one would expect more sensoria than is characteristic of typical wingless individuals in at least some of the intermediates with only .01 wing development which presumably changed to the winged condition very late in their embryogeny. This condition is not met in any of the intermediates with .01 wing development, yet there are many of these.

The data on intermediates, if the order of determination is wings, ocelli, and wing muscle as proposed by Stiles (1938), permit the interpretation that the greater number of sensoria characteristic of the winged aphid is determined neither very late nor early, but somewhere between these extremes. Increased sensoria cannot be determined early in development or they would be found more generally in intermediates with degenerate wing muscle; neither does it seem probable that they are determined very late or some of the aphids with a low degree of intermediacy (little development of wings) and non-degenerate muscles would possess intermediate antennae. With the exception of one, the most muscle found in intermediates with degenerate wing muscle is .57

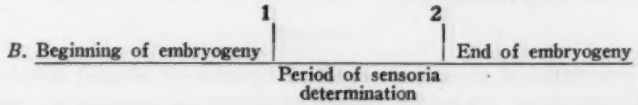
of normal development, indicating a change to wingless before the late stages in the segregation of this organ. The one exception of much muscle associated with degeneracy is P–27 of Table V. Unfortunately the sensoria number and antennal color of this aphid are not known as it was selected for a typical winged aphid and it was only after a histological study that intermediacy was recognized. If the antennae had been the light color of wingless aphids, it would seem as though that feature would have been observed, although it could have been overlooked for they were not examined with a microscope. Of those aphids in this investigation which were rated as to antennal characters and in which there were degenerate wing muscles, it may be postulated that development in the direction of wings was not far enough advanced before a change to wingless occurred for increased sensoria to be determined. All aphids in which the non-degenerate muscles are correlated with a low degree of wing development and a small amount of wing muscle are individuals in which the change to wings came too late in their embryonic development for an increase in sensoria to be determined. Only intermediate-winged aphids which change from a wingless to a winged condition early enough in their embryogeny to have wing muscles comparatively well developed pass through that part of the developmental period in which an increased number of sensoria is determined. This hypothesis may be illustrated by using a horizontal line to represent the entire embryogeny with vertical lines intersecting at various points to set off periods of time, as below.

Embryonic order of determination—wings, ocelli, wing muscle.

Aphid begins embryogeny wingless and changes to winged.



Aphid begins embryogeny winged and changes to wingless.



In *A*, intermediates with a small amount of wing muscle change to the winged condition at a time later than the interval marked off by the perpendicular line number 2, and therefore do not possess more sensoria than the typical wingless aphids. In *B*, intermediates with degenerate muscles as a consequence of a winged-to-wingless transition, change to

wingless before the time interval designated by 1 has been reached, and therefore do not possess an increased number of sensoria. Of the aphids in this study only those which changed from wingless to winged early enough to have considerable wing muscle passed through that part of the developmental period between the intervals 1 and 2.

These studies have led to the inference that the wings, ocelli, and wing muscles have their determination spread over considerable time, whereas the determination of the sensoria takes place in a relatively short time. An extensive series of intergradations in the development of the wings, ocelli, and wing muscles as contrasted with the decidedly limited series of the sensoria suggests this. From this analysis one concludes that increased sensoria are not determined until after the initial determination of wings, ocelli, and wing muscles; the former, however, complete their segregation before the latter.

From the data, it is concluded that the reason why aphids of this study with degenerate wing muscles did not usually possess increased sensoria is because the development in the direction of wings stopped short of the time when a greater number of sensoria would have been determined. Intermediates with considerable non-degenerate wing muscle changed from the wingless to the winged condition early enough to include in their development the period over which increased sensoria were determined. This analysis does not involve the inference that sensoria develop as a response to wing muscle, for in many cases the data show considerable wing muscle development without an intermediate number of sensoria. However, it is assumed that the time when increased sensoria in the embryogeny of the winged aphid are determined is indicated by the quantity of wing muscle. With the knowledge of the time when wing muscle is segregated in embryonic development as a point of reference, the time when sensoria characteristic of the winged aphid are determined can be ascertained provided that differentiation of the sensoria follows determination closely. According to this analysis, sensoria typical for winged forms are determined after wings, ocelli, and wing muscles.

Summary

Dark antennal color and increased sensoria of winged aphids are considered to be characters closely correlated in development, for in general when there was a darkening of antennae there was a corresponding increase in the number of sensoria. In practically all cases increased sensoria were correlated with a relatively large amount of non-degenerate wing muscle. It is concluded that embryonic determination of dark antennal color and increased sensoria takes place in a comparatively

short period of time as compared with that of wings, ocelli, and wing muscle. The data make it seem probable that dark antennal color and extra sensoria, characteristic of the winged aphid, are determined after the wings, ocelli, and wing muscle; the former, however, complete their segregation before the latter.

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SOME PHYSIOLOGICAL PROPERTIES OF DEXTRAL AND OF SINISTRAL FORMS IN BACILLUS MYCOIDES FLÜGGE

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The attention of biologists has long been concentrated on the study of dextral and of sinistral forms in populations of the same species. Recently Ludwig (1932, 1936) in two extensive reviews has summarized the numerous scattered observations in this field. Among studies of dextrality and sinistrality in the broad sense it seems useful to treat separately a distinct group of observations on spiral structures which are spread widely in different representatives of both plant and animal kingdoms. One of the basic attributes of spiral structures is their property of undergoing genotypic inversion with comparative ease, as has been shown, for instance, by Boycott, Diver, Hardy and Turner (1929) in the mollusk *Limnaea peregra*. In almost all cases when experimental work is carried out with organisms possessing a spiral form, it is possible to detect among the individuals of typical structure a few hereditary inverse specimens.

It may be supposed that the direction of the spiral is determined by some substance which is labile in the sense that it is able to undergo inversion of configuration with comparative ease. Such an idea is due to Koltzoff (1934) and to Needham (1934), who wrote that it is possible that the origin of dextrality and sinistrality in the eggs of certain snails, which later appear in the direction of spiral twist of their coil, is due to sterochemic properties of the protein molecules composing them. At present these relations are very obscure, and it seems essential to record accurately which physiological properties of dextral and sinistral spiral forms of organisms are identical and which are not. Such analysis is a necessary step in the attempt to comprehend the mechanism of morphologic inversions.

The Material and its Morphologic Properties

For the investigation of physiological peculiarities of dextral and sinistral spiral forms *Bacillus mycoides*, described by Flügge in 1886, was chosen. Various cultures of dextral and sinistral forms of this bacterium have been received from the Microbiologic Institute of the

Academy of Sciences (Moscow). Under natural conditions a form of *Bacillus mycoides* is usually met with which, when grown on the surface of agar-peptone medium, produces colonies spirally twisting to the left, i.e., counter-clockwise. The inverse form of this microbe, producing dextral coils in growing, rarely occurs. Isolated colonies consisting wholly of dextrally twisted individuals are sometimes found in soils. The dextral form of *Bacillus mycoides* was first recorded by Gersbach (1922), and in experiments with agglutination he established that the dextral and sinistral strains of this organism are perfectly identical in all their properties. Later dextral strains among a number of sinistral ones were observed by Oesterle (1929) and by Lewis (1932). The dextrality and sinistrality in *Bacillus mycoides* is a hereditary feature inasmuch as dextral forms are always obtained from dextral forms and sinistral forms from sinistral after numerous transfers on peptone-agar.

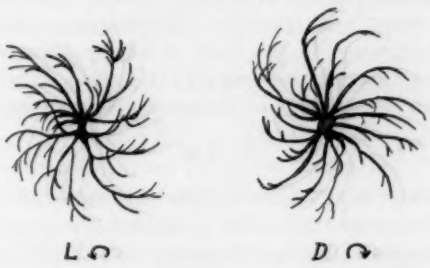


FIG. 1. Dextral (*D*) and sinistral (*L*) colonies of *Bacillus mycoides*, 18 hours after inoculation on peptone-agar in Petri dishes, at 29° C. Diameter of the colonies attains 20 mm.

Figure 1 represents the appearance of colonies formed by the dextral and sinistral forms of *Bacillus mycoides* on peptone-agar. With the introduction of a small quantity of inoculating material into the centre of a Petri dish of agar-peptone, the growth begins and the thin filaments of the growing culture of the L-form steadily deviate to the left and those of the D-form to the right.

It can be shown that the spiral form of the colony of *Bacillus mycoides* is a secondary feature which is the result of the primary spiral structure of the growing cells of the filament. If one stains the growing cells of the filaments on the surface of agar by neutral red or by toluedin blue (1 : 5000), and then examines them under the microscope, one sometimes detects the twisting of two filaments on encountering one another on the surface of the agar. A number of such observations prove that during the growth of the filament motions of two kinds take place simul-

taneously: first, the elongation and, second, the twist around the axis of the filament. As a result of the combination of these two motions, the structure of the individual cell and with it the structure of the whole filament forms a spiral. Similar observations have been made also by Stapp and Zycha (1931) and by Roberts (1938).

If during the free growth of a filament on the agar surface, the filament rotates around its longitudinal axis counter-clockwise, as a result of interaction with the firm surface of the agar the growing filament will also steadily coil in a counter-clockwise direction. Consequently, the secondary sinistral coil of the growing colony of bacteria will arise as a result of the primary sinistral spiral growth of the cells of the filament. This viewpoint is confirmed by the following observations. It appears that a certain consistency of culture medium is necessary for the typical spiral growth of colonies of *Bacillus mycoides* (Pringsheim and Langer, 1924; Hastings and Sagen, 1933). The latter authors state that on agar of usual strength the growth of *Bacillus mycoides* spreads from the place of inoculation in the form of coarse filaments which twist counter-clockwise, forming a symmetrical pattern. On less consistent agar this pattern disappears, and the growth becomes diffuse.

Methods

The strains used in this investigation were cultivated during the last five years under standard laboratory conditions. It is recorded that long cultivation and repeated transfer of the strains of *Bacillus mycoides* level many differences in morphological and physiological characteristics observed immediately after the isolation of the strains from natural conditions (Kononenko, 1935), and only those of them are retained which are due to the hereditary pattern of organization of the strain. This circumstance is of significance in the comparison of physiological properties of dextral and sinistral forms.

Experiments were made under sterile conditions. The bacteria were cultivated in Petri dishes on a solid medium of the following composition: K_2HPO_4—0.05 per cent; $MgSO^4$—0.03 per cent; NaCl—0.03 per cent, peptone—1 per cent and agar-agar—2 per cent. In some experiments the effect of optical isomers of amino acids upon the bacteria was studied.[1]

[1] In this work preparations of the following origins were used: *d*-valine, *l*-valine, *l*-aspartic acid and *dl*-aspartic acid: Schuchardt Co. (Görlitz); *d*-leucine (reinst), *l*-leucine (reinst), *l*-histidine monochlorhydrate, *d*-histidine monochlorhydrate, *l*-β-phenyl-α-alanine (reinst), *d*-β-phenyl-α-alanine (reinst): Fraenkel and Landau (Berlin); *d*-arginine monochlorhydrate (recrystallized from 80° alcohol), *dl*-arginine monochlorhydrate (isolated by Dr. M. Lissitzin): Protein Laboratory of the Academy of Sciences (Moscow).

Relations of Growth in Dextral and in Sinistral Strains to Temperature

The first group of experiments was devoted to the study of the effect of temperature on the growth of dextral and sinistral strains of *Bacillus mycoides*. Before the inoculation *Bacillus mycoides* was always cultivated on solid agar in Petri dishes at 20° C., while for the last forty-eight hours the culture was maintained at 15° C. From such cultures, accustomed to moderate temperatures, an inoculation into solid or liquid medium was made and the growth was followed usually at the following temperatures: 20°, 24°, 28° and 32° C. In cases when it is not specially mentioned, the experiments were made with the sinistral strain, L–1, and with the dextral strain, D–1. The basic conclusions arrived at in the work with these strains were further confirmed upon other strains, as will be described below.

Figure 2 gives the results of three series of experiments made in Petri dishes. Into the centre of each dish was introduced a small quantity of inoculating material which formed a circle with a diameter of 0.5 mm. Twenty hours after the beginning of the growth the diameter of the colony was measured and as a rule it had attained about 6 mm. at 20° C., and about 25 mm. at 32° C.

Observations have shown that at this time the growth proceeds at all temperatures according to a geometric progression. In Fig. 2 the size of colonies of the dextral and sinistral strains growing at 20° C. was taken as a unit, and the size at other temperatures is expressed in relative values. The fundamental conclusion which may be drawn from Fig. 2 is that the sinistral and dextral strains of *Bacillus mycoides* differ sharply from one another in the relation of growth to temperature. If the rate of growth of the usual sinistral strain increases exponentially with the rise of temperature, as is typical for the majority of biological processes, in the inverse dextral strain the phenomenon of "heat injury" may be observed. With the rise of temperature the velocity of growth of the dextral strain increases always more and more slowly.

This observation has necessitated a more thorough study of the phenomenon of "heat injury" in the dextral strain in additional material. In the preceding experiments the growth of the so-called R-forms of bacteria, producing sinistral (LR) or dextral coils (DR) was studied. As a result of "dissociation" both dextral and sinistral strains of *Bacillus mycoides* develop into smooth ones, the so-called S-forms, deprived of coiling and forming during their growth on solid medium flat spheric colonies (LS and DS) (for literature see Arkwright, 1930). In their external appearance S-forms of dextral and sinistral strains are certainly indistinguishable from one another. Investigations devoted to

the study of dissociation in *Bacillus mycoides* have shown that the transition of the R-form into S-form is very easily accomplished, while the reverse transformation of the S-form into the R-form is extremely

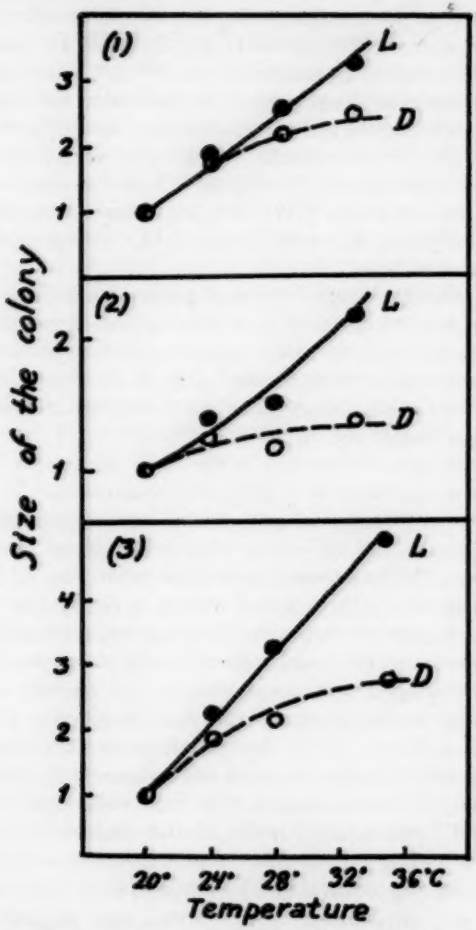


FIG. 2. Temperature relation of growth in the dextral (*DR*) and sinistral (*LR*) strains of *B. mycoides* on the solid medium. Each point represents an average for three dishes.

unusual (Stapp and Zycha, 1931; Lewis, 1932; Dooren de Jong, 1933).

An attempt was made to find out whether the specific differences in the dependence of growth on temperature in the dextral and sinistral

strains which were observed on the R-forms will also be maintained in the case of the S-forms. Experiments on the relation of growth to temperature in LS and DS forms of *Bacillus mycoides* growing on solid

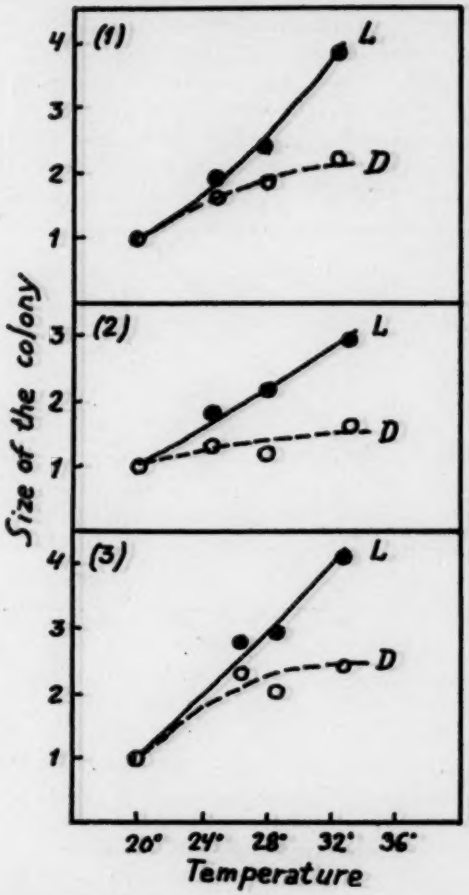


FIG. 3. Temperature relation of growth in the smooth dextral (*DS*) and sinistral (*LS*) strains of *B. mycoides* on the solid medium. Each point represents an average for three dishes.

medium were made according to the previous plan and the results of the three series of observations are given in Fig. 3. It is evident that the phenomenon of heat injury in growth which was found to be char-

acteristic for the dextral strains is practically equally marked both in the R-form and in the S-form of the bacteria.

Further experiments on the relation of growth to temperature in DS and LS strains were made with liquid medium of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent, glucose—1.0 per cent, d-arginine—0.05 per cent. The medium in this and in further experiments was sterilized without arginine, and a powder of arginine was aseptically added to it later. The bacteria were cultivated in test-tubes previously sterilized by dry heat and containing 1 cc. of aseptically added sterile culture medium. The inoculating material was prepared in the following manner. Two loops of

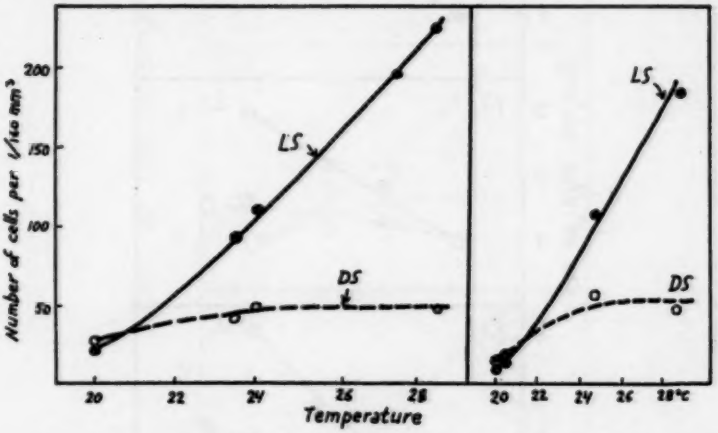


FIG. 4. Temperature relation of growth in the *DS* and *LS* strains of *Bacillus mycoides* in the liquid medium. Each point represents an average for four observations.

bacteria growing in Petri dishes on agar-peptone at 20° C. were shaken up in 3 cc. of sterilized bidistilled water, and two drops of this mixture were aseptically introduced into each of the experimental test-tubes, which were subsequently kept at different temperatures (20°, 24°, 26° and 28° C.). The measurement of growth consisted in counting the number of cells in a Thoma chamber under the microscope forty hours after the inoculation. In Fig. 4 the relation of growth to temperature is given as observed in two series of experiments upon the LS and DS forms of *Bacillus mycoides* in the liquid medium. It may be pointed out that in the liquid medium the velocity of growth of the sinistral strain rises exponentially with the increase of temperature, while in the dextral strain the characteristic heat injury is distinct in the range 24°–28° C

In the light of all these experiments it is certain that the dextral (D–1) and sinistral (L–1) strains of *Bacillus mycoides* under examination possess distinctly different relations of growth to temperature and that these distinctions are retained both in solid and in liquid medium, both in R and S modifications. However, are these observations sufficient for the belief that the differences observed are actually connected with the dextrality and sinistrality of the strains studied? In order to

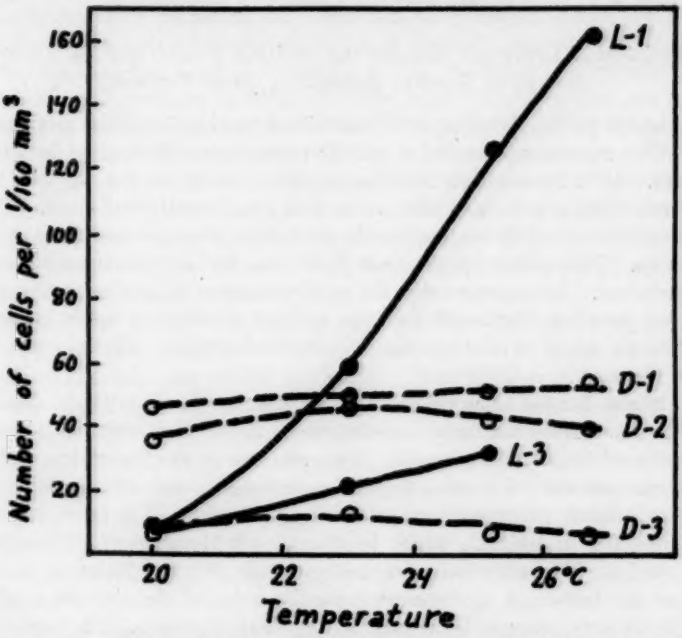


FIG. 5. Temperature relation of growth in the smooth sinistral (*L–1, L–3*) and dextral (*D–1, D–2, D–3*) strains of *Bacillus mycoides* in the liquid medium. Each point represents an average for three observations.

answer this question it is necessary to observe a great number of various strains of *Bacillus mycoides*. To this end one additional sinistral strain, L–3, and two new dextral inverse strains, D–2 and D–3, were chosen. All these strains had been cultivated under laboratory conditions for five years. S-modifications of various strains were used for experimentation for the reason that it is easier to prepare the suspension for inoculation from them. The results of experiments carried out in liquid medium with five strains of *Bacillus mycoides* of different origin are given in Fig. 5. It may be pointed out that the differences in the

relation of growth to temperature are more distinctly expressed in the two strains which were previously compared, i.e., in L–1 and D–1. However, if one examines the temperature range 23°–26° C., it may be observed that there is a specific distinction between all the dextral and all the sinistral strains. In dextral strains (D–1, D–2, D–3) in this temperature range the rate of growth does not rise with the rise in temperature, while in both sinistral strains (L–1 and L–3) the rate of growth rises considerably with the increase in temperature.

Physiological Difference between the Sinistral Strains and the Dextral Strains of Bacillus mycoides (Lewis Reaction)

Lewis (1933), working in Texas with dextral and sinistral strains of *Bacillus mycoides,* recorded a specific physiological distinction between them. It is known that *Bacillus mycoides* possesses the capacity of decomposing glucose and saccharose with the formation of acids. The production of acids on glucose is similar in sinistral and in dextral strains. This similarity does not hold true for the decomposition of saccharose. Lewis writes that the rapid formation of acid on saccharose is, on the whole, connected with the sinistral direction of spiral growth, while the arrest of this reaction is correlated with the dextral twist of the filaments.

It was decided to repeat these experiments of Lewis with the strains of *Bacillus mycoides* under investigation. S-forms of different strains were used in these experiments. Two per cent of saccharose was added to agar-peptone of usual composition and also some weak (0.02 per cent) solution of phenol red (according to Clark). The latter had an orange tint at pH 7.7, which is optimal for the growth of *Bacillus mycoides.* The microbes were cultivated at 28° C. Nineteen hours after the beginning of the experiment the color of the sinistral strains differed very sharply from that of the dextral strains. The sinistral strains, L–1 and L–3, showed rapid production of acid on sucrose. The reaction of the colony was distinctly acid to phenol red (pH about 6.8). The dextral strains, D–1, D–2 and D–3, showed no production of acid on sucrose. The reaction of the colony was alkaline to phenol red (pH about 8.4).

The results of Lewis are therefore confirmed and it may be pointed out that we used S-modifications while he used R-modifications of the bacteria. This confirmation includes also the following detail. The dextral strains are, without exception, unable to produce acid on sucrose, while almost all sinistral strains readily form acid on sucrose. To this general rule, Lewis noted one exception and I have also come across one doubtful case.

The reaction of Lewis may be considered to be a certain fermentative weakness of inverse dextral strains as compared to typical sinistral ones.

On the Relation of Morphological Inversion to Molecular Inversion

The phenomenon of heat injury of inverse dextral strains of *Bacillus mycoides* coincides in a remarkable way with the heat injury taking place on cultivating different lower organisms on unnatural isomers of amino acids described by Gause and Smaragdova (1938). When the yeast cells *Torula utilis* are grown on the usual isomer of leucine, which enters into the composition of all living organisms, the velocity of growth rises exponentially with an increase of temperature, but when they are cultivated on the unnatural isomer of leucine the velocity of growth increases always more and more slowly with the rise of temperature.

It was decided to repeat this observation of Gause and Smaragdova (1938) on different amino acids and on different organisms. The experiments were made on the growth of *Torula utilis* on optical isomers of leucine, hystidine, phenyl-alanine and valine. Cultivation took place in sterile test-tubes containing 1 cc. of liquid medium of the following composition: KH_2PO_4—0.06 per cent, $MgSO_4$—0.03 per cent, glucose —1.0 per cent, amino acid—0.4 per cent (the latter being aseptically introduced into the sterile medium). The inoculating material was taken from the solid medium, and from it was made a mixture of standard density (50 cells in 1/160 cu. mm.), a few drops of which was then added to liquid in the test-tubes. The counting of the number of cells in the Thoma chamber was made 40 hours after the beginning of the experiment. According to Gause and Smaragdova (1938) this time lies within the limits of the logarithmic phase of growth of *Torula.*

The results of our experiments are given in Fig. 6. Typical heat injury in the temperature range of 18°–20° may be observed in the growth of *Torula utilis* on unnatural isomers of leucine and of histidine. In the case of growth of unnatural isomers of valine and phenyl-alanine such injury is not observed.

Further experiments were made with the growth of *Aspergillus niger* on optical isomers of leucine and valine. The composition of culture medium was as follows: KH_2PO_4—0.35 per cent, $MgSO_4$—0.17 per cent, glucose—1 per cent, amino acid—0.4 per cent. Cultivation was conducted under sterile aerobic conditions in 2 cc. of the liquid. Seventy hours after the beginning of the experiment the wet weight of the mycelium of *Aspergillus,* which was previously drained on filter paper, was measured. The results of experiments given in Fig. 7 coincide completely with the observations made on *Torula utilis.*

Summarizing the results heretofore described, it is evident that during the growth of different lower organisms on optical isomers of certain amino acids the effect of temperature on the growth on natural and on

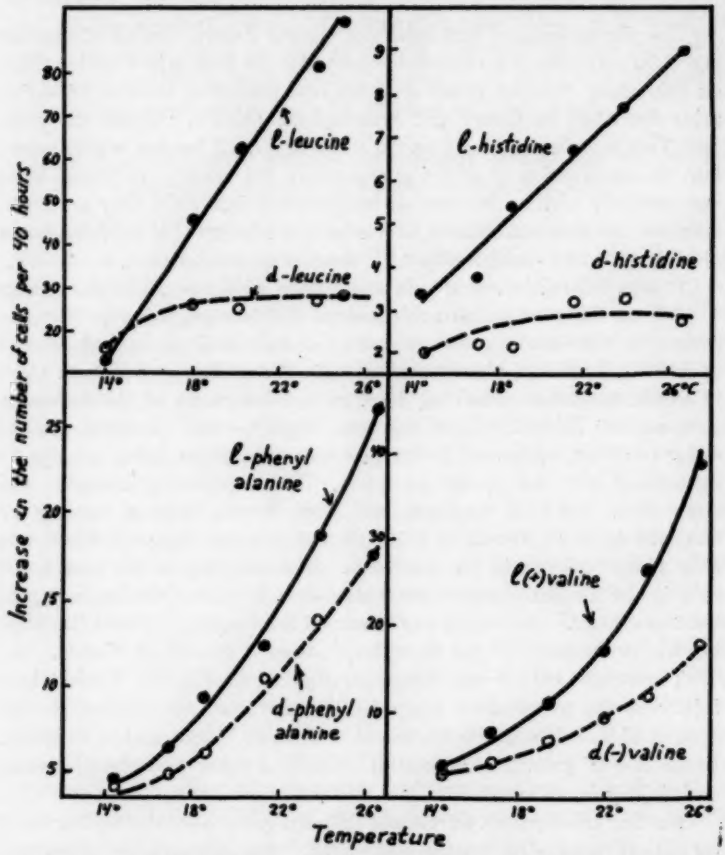


FIG. 6. Growth of the yeast *Torula utilis* on optical isomers of various amino-acids at different temperatures. On the ordinates is plotted the increase in the number of cells per 40 hours in relation to control (the initial density at inoculation, which is taken for a unit). Each point represents an average for two similar cultures.

unnatural isomers proves to be different. The velocity of growth on natural isomers rises exponentially with an increase of temperature. On the other hand, in the same temperature range, the velocity of growth

on the unnatural isomer of some amino acids increases with the rise of temperature always more and more slowly. A similar temperature-velocity curve was observed in the inverse dextral strains of *Bacillus mycoides* while they were being grown on the natural substrates. It follows from this type of curve that there is an inhibitive factor in growth and that the intensity of the inhibition rises with the rise of temperature.

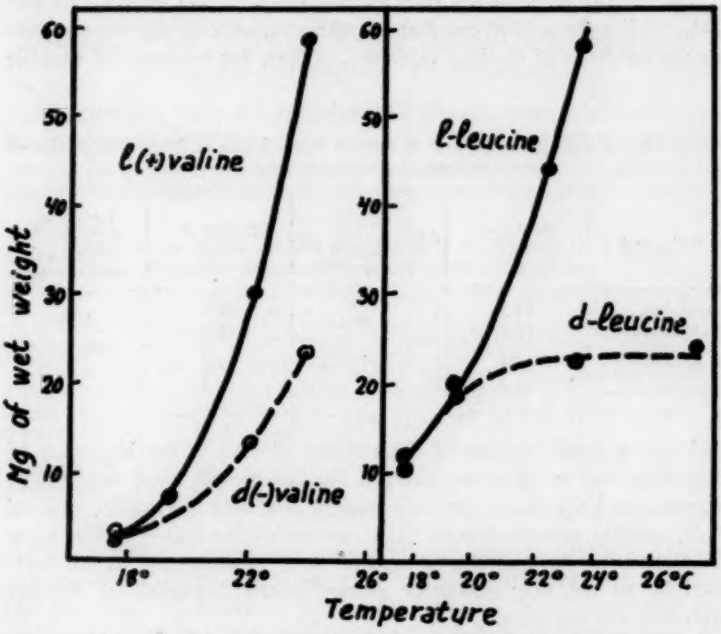


FIG. 7. Growth of *Aspergillus niger* on optical isomers of leucine and valine at different temperatures. Each point represents an average for two similar cultures.

It may be conjectured that in the first series of investigations the unnatural isomer of amino acid dissolved in the culture medium surrounding the cell causes a depression of growth because its steric configuration does not coincide with the steric configuration of the basic constituents of protoplasm. In the second series of investigations one may suppose that the depression of growth in the morphologically inverse form takes place because inside the cell there is an inverse of the normal optical isomer of some organic substance participating in the

determination of the form of cells. The depression of growth could thus take place because the steric configuration of the organic substance in question does not coincide with the steric configuration of other constituents of protoplasm.[2]

Growth of Dextral and Sinistral Strains of Bacillus mycoides on Optical Isomers of Certain Amino Acids

All further experiments were carried out with the strains L–1 and D–1. It is to be pointed out that not all amino acids by far are adequate for the nutrition of *Bacillus mycoides*. Thus, for instance, on aspartic

TABLE I

The growth of sinistral (LR) and of dextral (DR) forms of *Bacillus mycoides* on optically isomeric arginines.

Amino acid	Number of cells in LR	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit	Number of cells in DR	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit
d-arginine.....	31.3	1.99	19.7	1.59
dl-arginine.....	15.7		12.4	
d-arginine.....	28.9	1.98	21.2	1.61
dl-arginine.....	14.6		13.2	

acid only a small increase of the number of cells at the beginning of cultivation can be observed, and the further growth soon stops. The experiments have shown that on l-aspartic acid both the smooth sinistral (LS) and the smooth dextral (DS) strains of *Bacillus mycoides* grow better than on the racemic dl-aspartic acid. Among the amino acids used by us the only substance giving unlimited growth of *Bacillus mycoides* was arginine.

The experiments were made at 24° in test tubes with liquid medium of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent, glucose—1 per cent and arginine—0.05 per cent. Each number given in Tables I and II is a mean value for five parallel observations. In all cases the number of cells in 1/160 cu. mm. was counted 48 hours after the beginning of the experiment.

Both the dextral and sinistral forms of *Bacillus mycoides,* in both twisted and smooth modifications, grow better on natural d-arginine

[2] This hypothesis is further supported by recent observations of Bruckner and Ivanovics (*Zeitschr. f. physiol. Chem.*, **247**: 281, 1937). They observed that in the composition of the capsule of some bacteria an unnatural isomer of glutamic acid participates.

than on the racemic dl-arginine. Since racemic arginine in weak watery solution consists of the dextrorotatory and of the laevorotatory isomers, the lessened growth of *Bacillus mycoides* on this preparation may be explained by the fact that the unnatural laevorotatory isomer, entering into the composition of racemic arginine, is less appropriate for growth than the usual dextrorotatory isomer of arginine. In this relation the properties of dextrally and of sinistrally twisted strains coincide with one another.

Oxidation of Glucose by Dextral and Sinistral Strains of Bacillus mycoides at Different Temperatures

Experiments with the oxidation of glucose were made with the Warburg technique at three temperatures: 22°, 25° and 28° C. Bacteria growing on the solid medium at 20° were removed with a platinum loop

TABLE II

The growth of smooth sinistral (LS) and of smooth dextral (DS) forms of *Bacillus mycoides* on optically isomeric arginines.

Amino acid	Number of cells in LS	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit	Number of cells in DS	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit
d-arginine.....	40.1	1.26	66.8	2.15
dl-arginine.....	31.9		31.0	
d-arginine.....	43.5	1.87	45.5	1.38
dl-arginine.....	23.3		33.0	

and a suspension of them was made on a salt solution of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent. Experiments were made with 0.5 cc. of liquid and the oxidation of 0.5 per cent glucose was studied in the atmosphere of oxygen. Into the side tube of the Warburg vessel was poured a 5 per cent solution of KOH for the absorption of carbonic acid. The experiment at every temperature was always begun with a fresh suspension of the standard concentration. Five Warburg manometers were used simultaneously. One of them served as thermobarometer, two of them contained the suspension of sinistral (LS) bacteria, and two others the suspension of dextral (DS) bacteria. Fifteen minutes were allowed for temperature equilibrium, and then the readings of oxygen consumption were made at 20-minute intervals for 1½ hours. On the basis of these data the mean values of oxygen consumption at different temperatures were calculated.

Figure 8 gives the results of two series of experiments. As is characteristic of biological processes generally, the velocity of respiration rises exponentially with the rise of temperature, and practically in equal degree both in the sinistral and in the dextral strains of *Bacillus mycoides*.

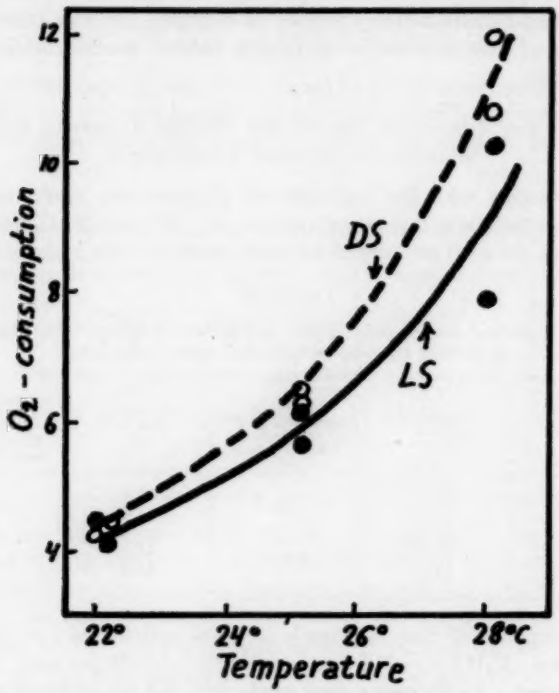


FIG. 8. Oxygen consumption by suspensions of dextral (*DS*) and sinistral (*LS*) strains of *Bacillus mycoides* at different temperatures. Oxygen consumption per 20 minutes is expressed in divisions of the standard manometer and represents an average for four consecutive readings.

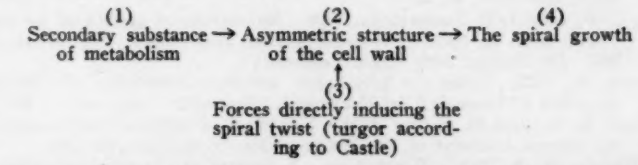
Consequently the phenomenon of heat injury in the dextral strain, which is characteristic for its growth, is absent in respiration on glucose.

DISCUSSION

Investigations of different physiological properties of dextral and of sinistral strains of *Bacillus mycoides* show that in the basic features of their metabolism these strains are alike. Both in the sinistral and in the dextral strains natural dextrorotatory arginine proves to be more

suitable material for growth than the racemic arginine. Both the sinistral and the dextral strains oxidize natural dextrorotatory glucose equally well and with the same temperature coefficients. The specific difference between dextral strains and sinistral ones is consequently connected not with the inversion of the fundamental protoplasmic system but with differences in some secondary substances determining the cellular form. Castle (1936) has recently undertaken the study of the spiral growth of the cell in *Phycomyces*. He advocates the idea that the spiral structure of the growing cell wall is not strictly predetermined beforehand, but depends on interaction of forces acting in the region of growth. The twist of the growing elastic elements of the wall may be the result of their resistance to the pressure of turgor, as has been shown by Castle (1936) with a special model. It is interesting that if the elastic elements of the wall are distributed symmetrically, in 50 per cent of the experiments with the model dextral spirals are obtained, and in 50 per cent sinistral spirals. As the sinistral direction of spirals is typical for *Phycomyces*, it is evident that the reason for this fact, if one adopts the explanation of Castle, is to be found in some sinistral distribution of elastic elements of the cell wall, which later, under the action of turgor, leads to the formation of sinistral spirals.

As Castle himself points out, the mechanism of spiral growth in different organisms can be different, and one cannot directly transfer his considerations to the bacteria, the more so since the cell wall of the latter consists of some specific protein material which is formed in close connection with the cellular protoplasm (John-Brooks, 1930). It is only important to realize that in the spiral growth of the cell wall there is, first, a system of forces directly inducing the spiral twist (like the turgor in the model of Castle) and, second, that there is some preexisting asymmetric system (distribution of the elastic elements of the wall), the dextrality or sinistrality of which brings on the dextrality or sinistrality of the spiral growth. Whatever the actual mechanism of spiral growth may be, one can conceive the following general scheme of the formation of spiral structure of the cell wall:



One may conjecture that the inversion of the direction of spirals in *Bacillus mycoides* is related to optical inversion of some secondary substance in metabolism. The latter brings on the inversion of some struc-

tures in the cell wall, and during the interaction of these with the forces inducing the spiral twist the inversion in the direction of the spiral growth of the cells is brought about.

In conclusion it is to be pointed out that the optical inversion of the secondary substances determining cellular form is judged from indirect evidence, and it is consequently highly desirable to check this conclusion by direct chemical methods.

Summary

1. The investigation of different physiological properties of dextral and of sinistral forms of *Bacillus mycoides* shows that in the basic features of their metabolism these strains coincide with one another. Both in sinistral and in dextral strains the natural dextrorotatory arginine proves to be more suitable material for growth than the racemic arginine. Both the sinistral and the dextral strains oxidize the natural dextrorotatory glucose equally well and with the same temperature coefficient.

2. The specific difference between the dextral and the sinistral strains is not consequently connected with the inversion of the basic protoplasmic system, but with differences in some secondary substances determining the cellular form. On the basis of the study of the phenomenon of heat injury in the growth of morphologically inverse dextrally twisted strains of *Bacillus mycoides* the hypothesis is advanced that some organic substance, participating in the determination of the cellular form, has undergone an optical inversion of configuration in the dextral strains.

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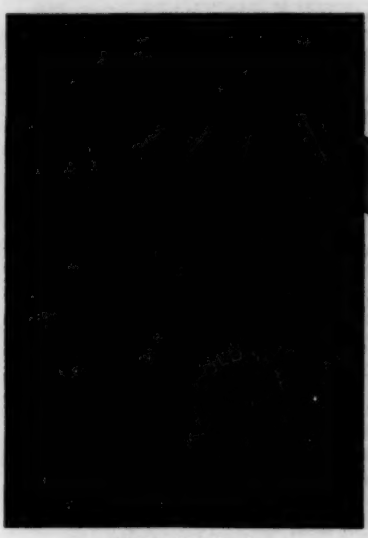

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